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(54) **Enzyme inhibitors.**

(57) Preparation of renin inhibitors based on the structure of natural renin substrate at residues 6 to 13 from the amino terminal thereof, the inhibitors being polypeptide analogues having in particular an isosteric non-peptide link corresponding to the 10, 11 peptide link of the substrate, and preparation of dipeptide analogues.

EP 0 045 665 A1

ENZYME INHIBITORS

The invention relates to renin-inhibiting peptide analogues.

BACKGROUND

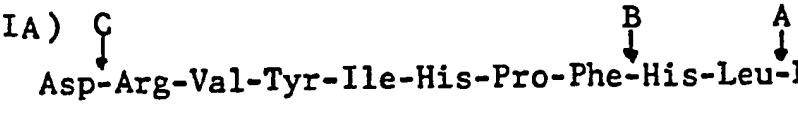
Renin is a natural enzyme, disorders in relation to which are implicated in many cases of hypertension. It is released into the blood from the kidney, and cleaves from a blood glycoprotein a decapeptide known as angiotensin-I. Circulating angiotensin-I is cleaved in lung, kidney and other tissues to an octapeptide, angiotensin-II, which raises blood pressure both directly by causing arteriolar constriction and indirectly by stimulating release of the sodium-retaining hormone aldosterone from the adrenal gland and thus causing a rise in extracellular fluid volume. The latter effect is caused by angiotensin-II itself or a heptapeptide cleavage product angiotensin-III.

Inhibitors of renin have therefore been sought, with two ends in view, first the provision of a diagnostic agent for identification of cases of hypertension due to renin excess, and secondly the provision of an agent for control of hypertension in such cases.

The present inventors' approach has been to consider the peptide sequence characterising the natural renin substrate at its binding site, and to seek peptide analogues sufficiently similar to bind to the enzyme, in competition with the natural substrate, but sufficiently dissimilar to it to be cleaved slowly or not at all. Such analogues will block the action of the enzyme and attack the hypertension at source.

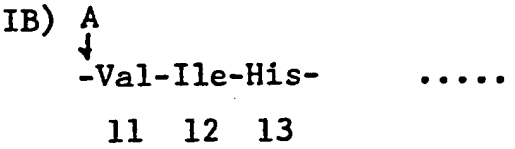
Renin is specific to a particular bond in the substrate, the N-terminal sequence of which in the horse is for example:

(IA)



as found by L.T. Skeggs et al J. Exper. Med. 106 439 (1957). Human renin substrate has a different sequence recently discovered by D.A. Tewkesbury et al Biochem. Biophys. Res. Comm. 99 1311 (1981)

(IB)

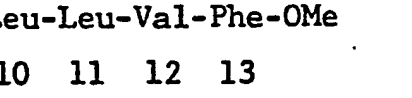


the sequence to the left of the arrow A being as in formula (IA).

Cleavage at A gives angiotensin-I; subsequent cleavage at the Phe-His bond at B gives angiotensin-II; and cleavage subsequently again at the Asp-Arg bond at C gives angiotensin-III.

Peptides similar to certain partial sequences of the substrate have been shown to act as inhibitors of renin in vitro. An example is the tetrapeptide ester (the relation to the substrate residues being indicated by numbering):

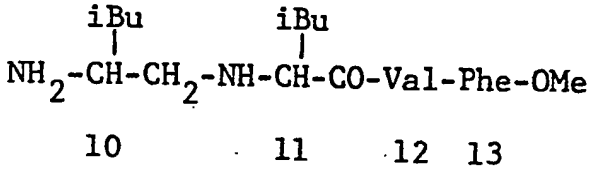
(II)



proposed by Kokubu, Nature, 217 456 (1968) but it is inactive in vivo, because of binding to plasma proteins and rapid attack by natural peptidases.

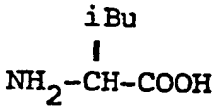
One of the present inventors undertook some years ago a development of Kokubu's work, seeking a renin inhibitor active in vivo, in which analogues of peptides similar to Kokubu's were made but having a methylene imino group $-CH_2-NH-$ in place of the peptide link -CO-NH- between the leucine residues. One of these analogues was:

(III)



which is the tetrapeptide (I) modified at the Leu-Leu link, leucine of course being

(IV)



This analogue (III) was the first effective in-vivo inhibitor of renin and was shown to have significant antihypertensive action in Goldblatt hypertensive rats (Parry, Russell and Szelke p. 541 in "Chemistry and Biology of Peptides" Ed. Meienhofer, Ann Arbor Science Publishers 1972). Little or no attention has however been paid to the work, which the authors themselves were unable to pursue, in spite of considerable activity in the general field of substrate-based inhibitors for renin, reviewed for example by Haber & Burton, Federation Proc. 38 No. 13 2768-2773 (1979).

THE INVENTION

The present invention is a development of the above work. Behind it is a concept of modifying peptide structures related to the peptide sequence at the site of action of renin on the natural substrate, by isosteric substitution at, at least, the site of cleavage. Optionally further there is isosteric substitution or other modification at other positions to increase stability or to modify the properties of the final peptide, for example its solubility under physiological conditions or its resistance to in vivo exopeptidase attack. Such modification may for example be by incorporation of residues other than those of the natural L-amino acids; by protection of the N-terminus with acetyl, pivaloyl, t-butyloxycarbonyl (Boc), benzoyl or other groups; or by conversion of the C-terminal carboxyl to another functional group, e.g. the corresponding alcohol, present as such or in ether or ester form.

General reference to amino acids and amino acyl residues and side chains in both the description and claims herein is to be taken as reference to such whether naturally occurring in proteins or not and to both D- and L- forms, and amino is to be taken as including imino except where an aromatic acid, residue or side chain is specified.

The compounds of the present invention, showing desirable renin inhibitory action, are of the general formula:

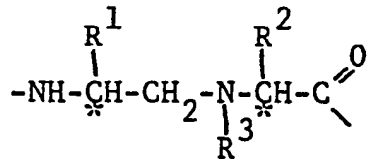
X-Y-Pro-Phe-His——A——B—Z—W (V)

6 7 8 9 10,11 12 13

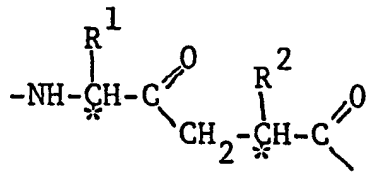
where Pro, Phe and His may be in substituted form;

X = H; or an acyl or other N-protecting group e.g. acetyl, pivaloyl, t-butyloxycarbonyl (Boc), benzoyl or lower alkyl (primarily C_1-C_5); or an L- or D-amino- acyl residue, which may itself be N-protected similarly;

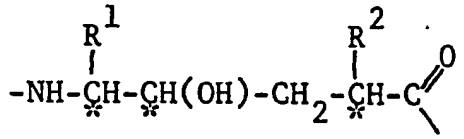
Y = D- or L-His or other D- or L- basic or aromatic amino-acyl residue, or is absent;

A =  (VI) "reduced" isostere bond

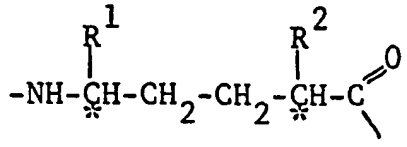
or

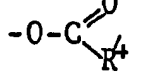
 (VII) "keto" isostere bond

or

 (VIII) "hydroxy" isostere bond

or

 (IX) "hydrocarbon" isostere bond

where the configuration at asymmetric centres * is either R or S, where in VIII the hydroxy group may be present as such or protected in ether $-OR^4$ or ester  form

where R^4 is as given under W below and where

R^1 and R^2, the same or different = ^{i}Pro (isopropyl),

^{i}Bu (isobutyl), Bzl (benzyl) or other lipophilic or aromatic amino-acid side chain

R^3 = -H; lower alkyl (C_1-C_5); or $-SO_2Ph$, $-SO_2C_6H_4CH_3(p)$, Boc, formyl or other N-protecting group;

B = D- or L- Val or Ile or other D- or L- lipophilic amino-acyl residue;

Z = D- or L- Tyr, Phe, His or other L- or D- aromatic amino-acyl residue;

and

W = -OH as such or in protected ester form as $-OR^4$ where R^4 = lower alkyl primarily C_1-C_5 and particularly ^{t}Bu, or cycloalkyl primarily C_3-C_7, or Bzl, or other ester forming group; or $-NH_2$ as such or in protected amide form as $-NHR^5$ or $-N(R^5)_2$ (where R^5 = an N-protecting or other substituent group e.g. lower alkyl as for R^4 and $(R^5)_2$ = two such or e.g. cycloalkyl, primarily C_3-C_7) or as $-NH-(CH_2)_n-Q$ or $-NR^5-(CH_2)_n-Q$ (where n = 2 to 6 and Q = NH_2 or

and wherein any of the hydrogens attached to nitrogen may be substituted by R^5 or $(R^5)_2$);

an L- or D- serine or lysine, arginine or other basic amino-acyl residue as such or in amide form substituted amide form or ester form e.g. containing a group or groups as given for R^4 and R^5 above as the case

may be; or an amino alcohol residue derived therefrom as such or protected in ester or ether form e.g. containing a group as given for R^4 above

or

Z + W = an alcohol derived from L- or D-Tyr, Phe, His or other L- or D- aromatic amino-acyl residue as such or protected in ester or ether form as above;

such polypeptide being in the above form or modified by isosteric replacement of one or more remaining peptide bonds by reduced, $-CH_2-NH-$, keto, $-C(=O)-CH_2-$, hydroxy, $-CH(OH)-CH_2-$, or hydrocarbon, $-CH_2-CH_2-$ isosteric links and further being in free form or in protected or salt form at one or more remaining peptide, carboxyl, amino, hydroxy or other reactive groups, in particular as their physiologically acceptable acid addition salts at basic centres.

The above compounds may in particular be those related to the substrate sequence in the horse (B = Val at position 12) or those related to the substrate sequence in man (B = Ile at position 12). Particular groups of these compounds are set out in claims 2 and 3 respectively herein, as formulae VA and VB to which reference may be made but which are not repeated at this point.

The numbering of residues in formulae (V), (VA) and (VB) shows the correspondence with the renin substrates themselves, but without limitation of the generality of the formulae.

Where a peptide bond in addition to that corresponding to the Leu-Leu or Leu-Val bond in the natural renin substrate is isosterically substituted, the 7,8 and 8,9 positions i.e. the Pro-Phe and Phe-His bonds in formula V are preferred,

or possibly both of these positions, and it is further preferred that the substitution should be

$$-CH_2-\underset{\displaystyle R_3}{\overset{\displaystyle |}{N}}- \qquad (X)$$

where R_3 is as set out above. The alternative isosteric substitutions set out herein may however be used.

Protective or substituent groupings as mentioned above may be any of these known in the polypeptide art, amply disclosed in the literature and not requiring discussion at length here. Generally the selection of the 'protective' groups is according to their function, some being primarily intended to protect against undesired reaction during synthetic procedures while the N- and C- terminal substituents are for example directed against the attack of exopeptidases on the final compounds or to increase their solubility and hence physiological acceptability.

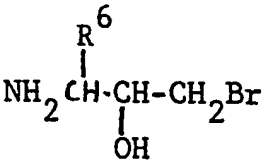
It is in particular possible for one or more remaining peptide bonds in the compounds of formula (V), (VA) or (VB) to be N-substituted with protective groups.

The invention further lies i) In a diagnostic test for high renin states, blood pressure falling most when renin is high, and as a surgical prognostic test for reno-vascular hypertension (renal artery stenosis), the administration of a polypeptide analogue as above followed by monitoring of blood pressure, and such polypeptide analogues when for such use, and

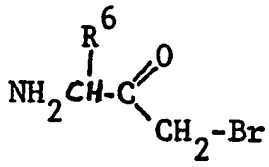
ii)In the long and short term treatment of heart failure and all forms of hypertension particularly those associated with high serum renin levels, the administration of a renin-inhibiting amount of a polypeptide analogue as above, and such polypeptide analogues when for such use.

The long and short term response of blood pressure to renin inhibitors is predictive of surgical outcome. In all cases single and repeated doses and any conventional form of pharmaceutical composition may be used, for administration by intranasal or oral route, injection, or any other means as convenient. Amounts may for example be 0.001 to 10 mg/kg body weight daily more usually 0.01 to 1mg, according to the potency of the analogue and the severity of the condition. Dosage unit compositions may contain such amounts or submultiples thereof to make up the daily dose. (Dosages herein and in the claims are related to the free base content where compounds are in salt form.)

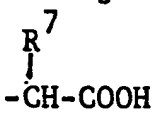
The invention still further extends to a product and method of making a hydroxy or keto isostere of a dipeptide wherein a derivative of a halohydrin preferably a bromohydrin or haloketone preferably a bromoketone



or



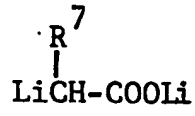
wherein R^6 is an amino acid side chain and the NH_2 and OH groups are in protected form is subjected to an alkylation procedure to attach a group



and gives the desiredisostere as such or in protected form, R^7 being the same or a different amino acid side chain.

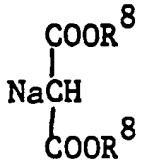
In particular the alkylation procedure may be

i) by reaction with an alkali metal carboxylic acid derivative preferably a lithium derivative



where R^7 is as above.

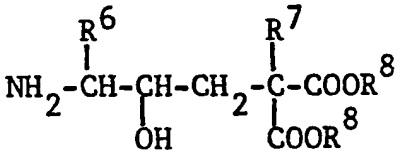
ii) by reaction with an alkali metal malonic ester derivative preferably a sodium derivative



where R^8 is an esterifying group and a halide preferably an iodide

$$R^7\text{-}I$$

where R^7 is as above to give intermediate



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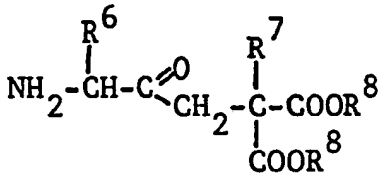
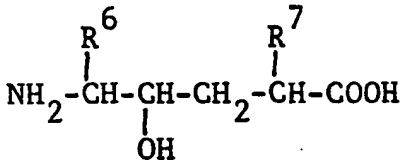


in protected form which intermediate is then decarboxylated and if desired deprotected to give the desired isostere



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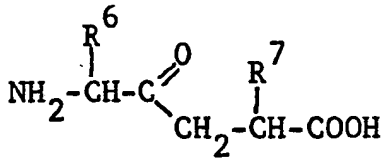
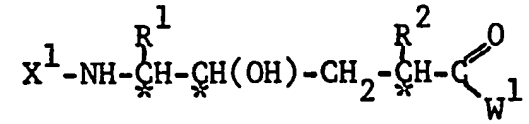


as such or in protected form.

The hydroxy isosteres so produced may further be oxidised to the corresponding keto isosteres, and such method and the isosteres produced fall within the invention.

In particular the methods may be applied to the production of a hydroxy dipeptide isostere of the formula



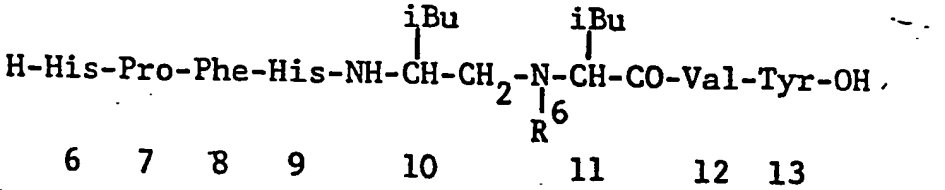
or the corresponding keto isosteres, where the significance of *, X^1 and W^1 is as above except that X^1 and W^1 do not represent amino-acyl.

The dipeptide isosteres given by all these methods may be incorporated in higher peptide analogues by the methods herein described or by the methods of peptide synthesis as generally known in the art, and the invention extends to the dipeptide whether as such or in the form of said higher analogues, in all cases as the compound itself or in protected form.

analogue

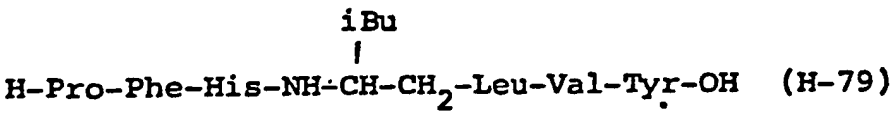
The dipeptide/syntheses are illustrated in detail herein, in the course of illustrating the preparation of the octapeptides and related compounds to which the invention chiefly relates.

Specific analogues within the invention, all as such or in protected form, are



(H-76 where R^6 = hydrogen; H-78 where R^6 = SO_2Ph)

and the corresponding analogue (H-77) with R^6 = hydrogen and D-His at position 6. A further analogue, with the same methylen -imino isost ric replacement of a Leu-Leu peptide bond, is:



Further analogues within formula (VA) are given in the present disclosure in Examples VI to IX, XI and XII. Analogues within formula (VB) are given in Examples V and X.

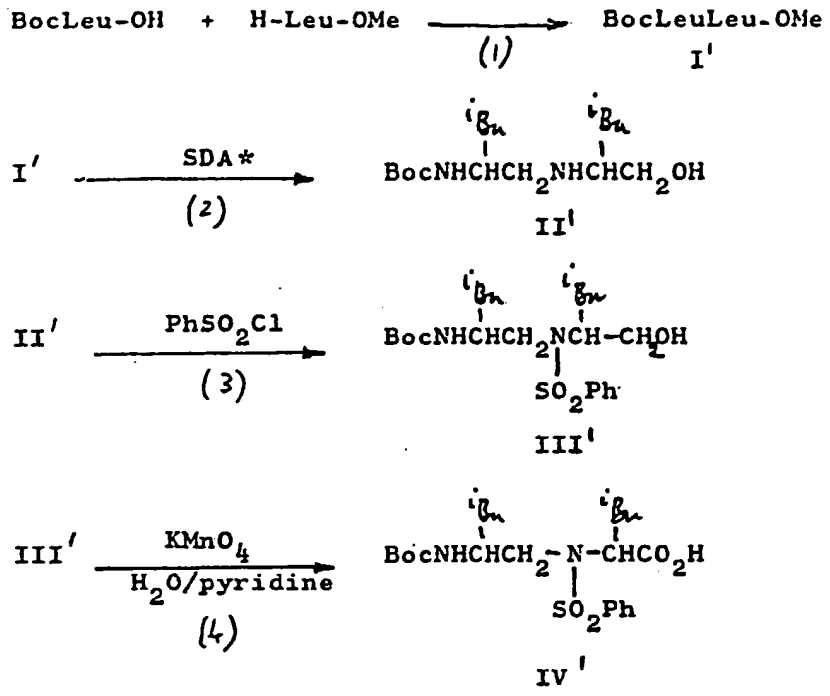
SYNTHETIC METHODS

The inventors have developed synthetic methods for the isosteric replacement of the peptide bond -CO-NH- with alternative groups, specifically $-CH_2-NH-$ (reduced), $-CH_2CH_2-$ (hydrocarbon), $-C(=O)-CH_2-$ (keto) and $-CH(OH)-CH_2-$ (hydroxy) isosteres (see, e.g. Szelke, et al, pp. 57-70 in "Molecular Endocrinology" Vol. 1, Editors: MacIntyre and Szelke, Elsevier, Amsterdam 1977, and Hudson, Sharpe and Szelke, U.S. Patent 4 198 398 "Enkephalin Analogues").

Reference may be made to these publications for general discussion of such isosteric replacement. A reaction sequence for the preparation in particular of the reduced isostere of leucyl leucine for incorporation in the analogues disclosed herein is however for example:

Scheme 1

Synthesis of the protected reduced isostere of L-leucyl-L-leucine



* Sodium di(methoxyethoxy) aluminium hydride

(1) Boc-Leucyl-leucine methyl ester

The dipeptide I' was prepared from Boc-leucine.H_2O (27.5g, 0.11 mole) and leucine methyl ester .HCl (20g, 0.11 mole) by mixed anhydride coupling using N-methyl morpholine and isobutylchloroformate. After a standard work-up procedure the dipeptide I' was obtained as white needles, 35.0g (88%) from EtOAc/petrol bpt 40-60°, m.p. 132-133° .

(2) Preparation of compound II'

The dipeptide I' (7.2g, 20 mmole) was dissolved in benzene (120 ml, Na-dried). A solution of sodium dihydro-bis-(2-methoxyethoxy)aluminate (SDA, 70% in toluene, 41 ml) was added slowly with cooling. After addition, the solution was refluxed for ½ hr, cool d and poured into 0.5M ice-cold citric cid solution. At pH 2.5 the aqueous solution was extracted with ether (4X) and the combin d extracts were discarded. The

pH was adjusted to 9 with Na_2CO_3 solution and the aqueous solution was saturated with sodium chloride. Extraction with ether (4X), followed by drying (Na_2SO_4) of the combined organic phases, evaporation and crystallisation from petrol (40-60°) at -20° gave the reduced dipeptide II' : 5.1g (78%) as white needles.

m.p. 59-60°

Nmr ($CDCl_3$) 9.05 - 9.15 (12H, d, 4 x $\underline{CH_3}$)
8.75 (6H, m, 2 x $(CH_3)_2\underline{CH}$-$\underline{CH_2}$); 8.55 (9H,s,$(\underline{CH_3})_3CO$);
7.35 (5H,m, $\underline{CH_2}NH$, $\underline{CH_2}OH$) ; 6.05 - 6.85 (3H,m, 2 x α-$\underline{CH}$ and $CH_2\underline{OH}$); 5.3 (1H, d, Boc $\underline{NH}$-).

(3) Protection of compound II' with benzenesulphonyl.

The reduced compound II' (11.0g, 34.7 mmole) in dioxan (100ml) was added to a solution of $KHCO_3$ (21g., 6 equiv.) in H_2O (100ml). This mixture was cooled in ice and benzene-sulphonyl chloride (9.0ml, 2 equiv.) added in dioxan (25 ml) with vigorous stirring. Stirred at 22° overnight. Poured into ether, washed with 2N NH_4OH (4X), H_2O (1X) 0.5M. citric acid (2X to remove any unsulphonated material), H_2O (1X).

The protected compound III' was obtained as an oil. Nmr spectroscopy showed the presence of one benzenesulphonyl group. This material was used without further purification in the next stage:-

(4) Oxidation of compound III'

The material from the preceding preparation was taken up in pyridine (50ml), cooled in ice and $KMnO_4$ (11.0g 70 mmole) in H_2O (50 ml) and pyridine (100 ml) added. Stirred for 42 hrs at 20°. The MnO_2 precipitate was removed and the filtrate diluted with citric acid solution until acidic. Ether xtraction at pH 5 r moved product and starting material. The product IV' was obtained by (i) $NaHCO_3$ extraction - to r move strongly acidic

by-products (ii) extracted with 30% v/v 0.880 ammonia solution (6X). The ammonia washes contained essentially pure IV. Starting material remaining in the ether was re-oxidised for 42 hrs and worked-up as above.

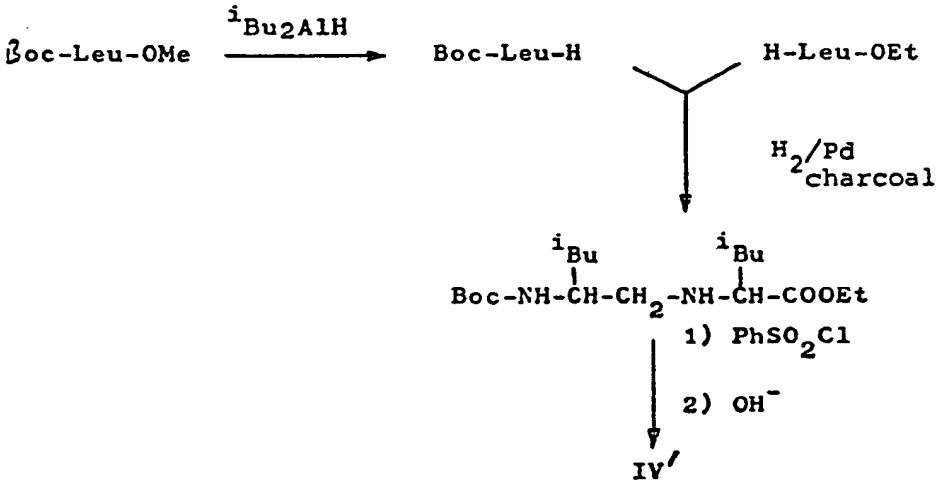
The total amount of IV' (isolated by acidifying the ammonia washes and extracting with $CHCl_3$) obtained was 2.34g (20% based on II').

The material was a colourless foam Rf 0.41 by TLC on silica in benzene-dioxan-acetic acid (95:25:4).

Nmr ($CDCl_3$): 8.9 - 9.3 (12H, m, 4 x $\underline{CH_3}$); 8.2 -8.8 (15H, m, $(\underline{CH_3})_3CO$ and 2 x $(CH_3)_2\underline{CH}$-$\underline{CH_2}$); 5.4 - 7.0 (4H, m, 2 x α-$\underline{CH}$ and -$\underline{CH_2}$-N-); * 2.0 and 2.4 (5H,m, $C_6\underline{H}_5SO_2$), 1.2 (1H,br.s, $CO_2\underline{H}$).

Alternatively, the reduced Leu-Leu analogue IV may be synthesised by the following method:

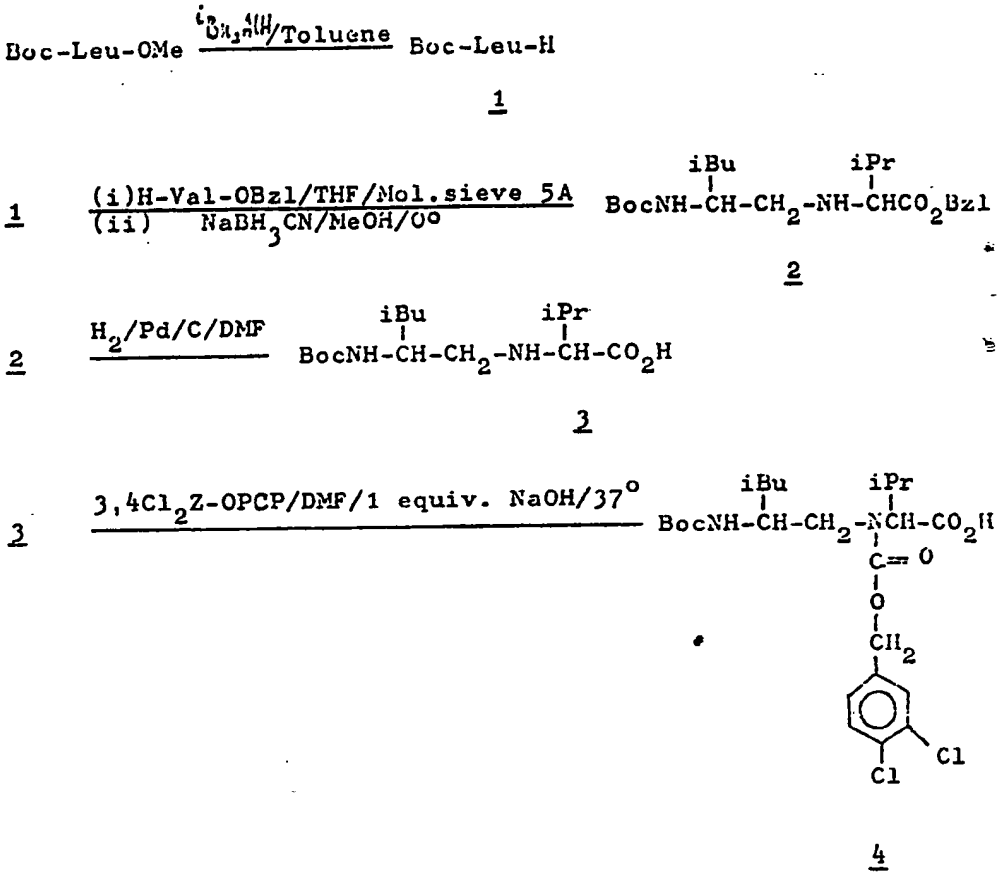
SCHEME 2



The following is a synthetic method f r a reduced Leu-Val isostere by a preferred route.

Scheme 3

Synthesis of the Protected Reduced Isostere of L-Leucyl-L-Valine



(1) Boc-L-Leucinal, 1

Boc-L-Leucine methyl ester (22.7g, 90 mmoles) in dry toluene (250ml) under N_2 was cooled to -78° and 25% di-isobutyl-aluminium hydride in toluene (130 ml, 225 mmoles) were added over 25 mins. keeping the temperature under -70°. The mixture was stirred for 15 mins. at -78° after completion of the addition, then MeOH (10ml) was added cautiously. When effervescence ceased the mixture was poured into an ice-cold solution of Rochelle salt (100 ml of saturated solution + 600ml H_2O). This mixture was shaken until an extractable solution was obtained. The toluene was separated and the aqueous phase re-extracted with ether (2 x 300ml). Toluene and ether

oil was passed rapidly through a pad of silica gel in 15% EtOAc/petrol 40-60°. The crude aldehyde was obtained as an oil, weight 18.68g. Nmr showed aldehyde content to be 85%, therefore yield of aldehyde: 15.9g (83%).

Nmr ($CDCl_3$), τ :

0.45 (1H,s,CHO); 4.87 (H,br.d.,Boc NH):

5.83 (1H,br.m., NH-CHCHO); 8.43-8.93 (12H,m,$(CH_3)_3C$, $(CH_3)_2CH.CH_2$); 9.0 and 9.1 (12H, 2 x d, $(CH_3)_2CH$)

TLC: (solvent 30% EtOAc/petrol 60-80°), Rf = 0.43.

(2) Boc-L-Leucyl-L-valine benzyl ester reduced isostere, 2

L-Valine-OBzl (10 mmoles, from EtOAc/1N $NaHCO_3$ partition of 3.8g of p-toluene sulphonate salt) and Boc-L-Leucinal (2.54g, 10 mmole aldehyde content) in dry tetrahydrofuran (20ml) stood over 5Å molecular sieve (10g) overnight. Sodium cyanoborohydride (630mg, 10mmoles) in MeOH (3ml) was added with cooling, then left at room temperature for 30 mins. The mixture was diluted with methylene chloride (100 ml), filtered and evaporated to dryness. The residue was passed down a silica column in 20% EtOAc/petrol (60-80°) to remove polar impurities. Isostere containing factions were combined. Crystallisation from petrol 60-80° at -20° gave large clusters of needles, 1.52g (38%) m.p.

τ : 2.65(5H,s,$OCH_2C_6H_5$);

6.35(1H,m,NHCHCO$_2$Bzl); 7.05 (1H,m,NH-CHCH$_2$);

7.45(2H,m, -CH$_2$NH-); 8.25 - 8.90(13H,m, $(CH_3)_3CO$-.) $(CH_3)_2$ CHCH$_2$ and $(CH_3)_2$ CH-); 9.05 and 9.15 (12H, 2 x s, 2 x $(CH_3)_2CH$).

TLC: (Solvent: 30% EtOAc/petrol 60/80°) Rf = 0.39

(3) N- (2S)-t-Butyloxycarbonylamino-4-methylpentyl, N-(3,4-dichlorobenzyloxycarbonyl)-L-valine, 4

Boc-L-Leucyl-L-valine, benzyl ester-reduced-isostere (1.5g 3.68 mmoles) in dimethylformamide (60ml) was hydrogenated at STP over 5% Pd/C (150mg). After 3½ hrs. the colloidal solution was flushed with nitrogen and 1M NaOH (3.8ml, 1.05 equiv.) was added followed by 3,4-dichlorobenzyl pentachlorophenyl carbonate (1.92g, 4.07 mmoles). The mixture was kept at 50° in a stoppered flask for 24 hrs. and then evaporated to dryness. EtOAc was added and the Pd/C filtered off. The EtOAc solution was washed with 1M citric acid (2 x), H_2O (1 x), brine (1 x), and dried (Na_2SO_4).

The crude isostere 4 was chromatographed on silica-gel (Merck Keiselgel 60, 40-63 m) eluting with 2% MeOH/$CHCl_3$ to give the title compound as a colourless oil.

Nmr($CDCl_3$), τ :

2.5 - 2.9(3H,m,$C_6Cl_2\underline{H}_3$); 3.3 - 3.8 (2H,br,BocN$\underline{H}$ and $CO_2\underline{H}$); 4.85 and 4.95 (2H,2 x s, $OC\underline{H}_2$-$C_6Cl_2H_3$): 5.5 - 6.3(2H,m,NHC$\underline{H}$CH and -NC$\underline{H}CO_2H$); 6.5 - 7.2 (2H br, 2 x d, CH$\underline{CH}_2$N-); 8.2 - 8.9 (13H,m,$(CH_3)_3CO$, $(CH_3)_2\underline{CHCH}_2$ and $(CH_3)_2\underline{CH}$-); 8.9 - 9.4 (12H,m,2 x $(CH_3)_2CH$)

TLC: (solvent 5% MeOH/$CHCl_3$) Rf = 0.32.

EXAMPLES

The following detailed Examples illustrate the invention.

The Examples are preceded by the preparation of Boc-Tyr [Bzl (2, 6 Cl_2)] -O-resin. (Reaction times marked * are convenient rather than necessary.)

Preparation of Resin

Boc-Tyr [Bzl(2,6 Cl_2)] -OH (1.65g, 3.75mmol) was dissolved in ethanol (20ml) and water (5ml) added. The pH was brought to 7.0 with cesium bicarbonate solution and the solvent evaporated in vacuo. The residue was treated twice with toluene and evaporated to remove the last traces of water leaving a white powder which was dried for several hours over phosphorus pentoxide. The residue was dissolved in DMF (65 ml), chloromethylated resin (10g, 7.5 mequiv.) added and the reaction stirred at 37° for four days.

The resin was then filtered and washed thoroughly with DMF, DMF/water (9:1) and then DMF again. The resin was then resuspended in DMF (65ml) and treated with acetic anhydride (2.36ml, 25mmol) and triethylamine (3.5ml, 25mmol) for 3 days.

Th resin was filter d, washed thoroughly with DMF, DMF/water (9:1) and methanol and dried. The resin was then "defined" by shaking a suspension in dichloromethane and removing the particles slowest to float. The resin was then

Yield 10.8 g.

Amino-acid analysis: (12N-HCl/propionic acid 1:1 130°, 2 hours) gave an incorporation of 0.11mmol/g.

Example I

H-His-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH (H-76)

Boc-Tyr [Bzl(2,6 Cl_2)] -O-Resin (3g, 0.6mmol) was washed with reagents in the following sequence: CH_2Cl_2 (3X) iPrOH (2X), CH_2Cl_2 (3X), 40% TFA/CH_2Cl_2 1 min then 20 min, CH_2Cl_2 (3X), iprOH (2X), CH_2Cl_2 (3X), 40% TFA/CH_2Cl_2 1min then 20 min, CH_2Cl_2 (3X) iPrOH (2X) CH_2Cl_2 (3X), 10% Et_3N/CH_2Cl_2 (2 x 2min), CH_2Cl_2 (5 X). Boc-Val-OH (0.65g, 3mmol) was then coupled using DCCI (0.68g, 3.3mmol) and HOBt (0.92g, 6mmol) in DMF/CH_2Cl_2 (1:1) for 17 hours. The resin was then washed with DMF (3X), CH_2Cl_2 (3X), iPrOH (2X), CH_2Cl_2 (3X) 10% Et_3N/CH_2Cl_2 (2 min), CH_2Cl_2 (5X) then acetylated using acetylimidazole (0.66g, 6mmol) in DMF for 1 hour. The resin was then washed with DMF (3X) CH_2Cl_2 (3X) iPrOH (2X) and finally CH_2Cl_2 (3X).

This sequence of washes and reactions was repeated for the addition of each of the residues with the following modifications.

After deprotection of the Boc-Val-Tyr [Bzl (2,6 Cl_2]-O-resin Boc-NH-CH (CH_2CHMe_2)-CH_2-N(SO_2Ph)-CH(CH_2CHMe_2)-CO_2H (0.42g, 0.9mmol) was coupled using DCCI (0.28g, 1.35mmol) and HOBt (0.275g, 1.8 mmol) in DMF/CH_2Cl_2(1:1) for 18 hours, foll wed by acetylation using acetylimidazole (0.66g, 6mmol) in DMF for 1 hour.

After deprotection,Boc-His(Dnp)-OH (1.44g, 3mmol) was coupled using DCCI (0.68g, 3.3mmol) and HOBt (0.92g, 6mmol) for 2 hours, followed by acetylation for 1 hour.

Deprotection of the histidyl peptide was achieved using 50 % TFA/CH_2Cl_2 instead of the usual 40% TFA/CH_2Cl_2. Boc-Phe-OH (0.796g, 3mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6mmol) for 17½ hours *, followed by acetylation for 1 hour.

Deprotection of the phenylalanyl peptide was achieved using the usual 40% TFA/CH_2Cl_2. Boc-Pro-OH (0.646g, 3mmol) was coupled using DCCI (0.68g, 3.3mmol) and HOBt (0.92, 6mmol) for 2 hours followed by acetylation for 1 hour.

After deprotection Boc-His(Dnp)-OH (1.44g, 3 mmol)was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6 mmol) for 14½ hours* followed by acetylation for 1 hour.

The resin was then washed with DMF (3X), CH_2Cl_2(3X) i PrOH (2X), CH_2Cl_2(3X) and finally MeOH (3X) and dried to give 3.5353g of product.

1.2g of this material was treated with HF at 0° for 1¼ hours in the presence of anisol (1.5ml) then dried overnight over potassium hydroxide. The resin was then washed with DMF/ water (1:1), acetic acid and finally acetic acid/water (1:1) to remove the peptide. These washes were combined and evaporated <u>in vacuo</u>.

The residue was dissolved in DMF (15ml) and water (6 ml) thioethanol (5ml) added and the pH of th solution brought to

8.0 with sodium carbonate. The reaction was stirred overnight the solvent evaporated and the residue applied to a Sephadex G 25 column (72 x 2.5 cms)eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 27-46 were combined and the solvent evaporated in vacuo and dried. Then 90% of the residue was taken (for the rest see Example III) and dissolved in anhydrous ammonia (100 ml) and small portions of sodium wire added until a permanent blue colour was achieved for 15 seconds. The ammonia was allowed to evaporate and the residue dried.

The residue was applied to a Sephadex SPC25 column (77 x 1.6 cms) eluted with 30 % acetic acid at 40 mls/hr, with a sodium chloride gradient from 0.01M to 1M over two days collecting 6.6 ml fractions.

The product was contained in fractions 100-104. These were pooled, evaporated and the residue dissolved in glacial acetic acid and filtered to remove the sodium chloride. The solution was evaporated and desalted on a Sephadex G25 column (72 x 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6ml fractions. Fractions 32-6 were pooled, evaporated, transferred to a vial and lyophilised.

Yield 13.4mg

Product $C_{52}H_{74}O_9N_{12}$ MW. 1011,25

T.l.c. (silica) Rf 0.15 EtOAc/Pyr/AcOH/H_2O 40:20:6:11

Rf 0.40 nBuOH/Pyr/AcOH/H_2O 30:20:6:24

T.l.e. pH 2.1 1000V 30 min mobility 8.3 cm.

pH 6.5 1000V 30 min mobility 7.5 cm.

AAA .6N HCl + phenol 110°, 40 hours, peptid content 72%

His:1.97; Pro:1.01; Val:1.02; Tyr: 0.98; Ph : 1.01.

Example II

H-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH (H-79)

Fractions 80-84 of the SPC 25 Sephadex column from the previous synthesis were combined, evaporated and the residue dissolved in glacial acetic acid and filtered to remove sodium chloride. The solution was evaporated and the product desalted on a Sephadex G25 column (72 x 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 32 - 9 were pooled, evaporated, transferred to a vial and lyophilised.

Yield 23.6 mg

Product	$C_{46}H_{67}O_8N_9$	MW 874,10		
Tlc (silica)	Rf 0.29	EtOAc/Pyr/AcOH/H_2O	40:20:6:11	
	Rf 0.46	nBuOH/Pyr/AcOH/H_2O	30:20:6:24	
Tle	pH 2.1	1000V	30 min	mobility 7.5 cms
	pH 6.5	1000V	30 min	mobility 8.3 cms

AAA 6N HCl + phenol, 110°, 40 hours, peptide content 85%

His: 0.97; Pro: 1.08; Val: 0.99; Tyr: 0.97; Phe:1.00.

The above example illustrates how Y in formulae (V), (VA) and (VB) may be absent.

Example III

H-His-Pro-Phe-His-Leu-reduced (SO_2Ph)-Leu-Val-Tyr-OH (H-78)

In the synthesis of compound H76 10% of the residue from the Sephadex G25 column after the HF and thioethanol treatments of the resin was kept.

This material was applied to a Sephadex SPC25 column (77 x 1.6 cm) eluted with 30% acetic acid at 20 mls/hr with a sodium chloride gradient from 0.01M to 1M over 2 days collecting 6.6 ml fractions.

The product was contained in fractions 74-7. These were pooled, evaporated, dissolved in glacial acetic acid and filtered to remove sodium chloride. The solution was then evaporated and desalted on a Sephadex G25 column (72 x 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 31-4 were pooled evaporated, the residue transferred to a vial and lyophilised.

Yield 0.6 mg

Product	$C_{58}H_{78}O_{11}N_{12}S$		MW:	1151.40
Tlc(silica)	Rf 0.31	EtOAc/Pyr/AcOH/H_2O		40:20:6:11
Tle	pH 2.1	1000v	30 min	mobility 5.4 cms.
AAA	6N HCl + phenol, 40 hrs, 110°, peptide content 64%			

His: 1.93; Pro: 1.08; Val: 1.05; Tyr: 0.96; Phe: 0.97.

Example IV

H-DHis-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH, (H-77)

The Boc-Tyr [Bzl(2,6,Cl_2)] -O-Resin (3g, 0.6mmol) was deprotected and Boc-Val-OH (0.65g, 3.0 mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6mmol) for 22 hours* then acetylated with acetylimidazole (0.66g, 6mmol) for 1 hour.

After deprotection, Boc-Leu-reduced (SO_2Ph)-Leu-OH, IV, (0.42g 0.9 mmol) was coupled using DCCI (0.28g, 1.35 mmol) and HOBt (0.275g, 1.8 mmol) for 20 hours, then acetylated for 1 hour.

After deprotection Boc-His(Dnp)-OH (1.44g, 3mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6 mmol) for 22 hours*, then acetylated for 1 hour.

After deprotection, this time with 50% TFA/CH_2Cl_2 Boc-Phe-OH (0.796g, 3 mmol) was coupled with DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6 mmol) for 19 hours* then acetylated for 1 hour.

After deprotection Boc-Pro-OH (0.646g, 3 mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6 mmol) for 3 hours then acetylated for 1 hour.

The peptide was again deprotected and coupled with Boc-D-His(Boc)-OH (0.91g, 2.56 mmol using DCCI (0.58g, 2.82 mmol) and HOBt (0.78g, 5.1 mmol) for 2 hours, then acetylated for 1 hour.

The resin was washed with DMF (3X) CH_2Cl_2 (3X) iProH (2X) CH_2Cl_2 (3X) and finally MeOH (3X) and dried to give 3.6563 g of product.

1.2g of this material was treated with HF at 0° for 1¼ hours in the presence of anisole (1.5 ml) then dried overnight over potassium hydroxide. The resin was then washed with DMF,

DMF/H_2O (1:1), acetic acid and finally acetic acid/wat r (1:1) to remove the peptide. These wash s were combined and evap rated in vacuo.

The residue was dissolved in DMF ($_{15ml}$) and water (6 ml), thioethanol (5ml) added and the pH of the solution brought to 8.0 with sodium carbonate solution. The reaction was stirred overnight. the solvent evaporated and the residue applied to a Sephadex G 25 column (72 x 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6ml fractions. Fractions 27-46 were combined and the solvent evaporated in vacuo and dried.

90% of the residue was dissolved in anhydrous ammonia (100 ml) and small portions of sodium wire added until a permanent blue colour was achieved for 15 seconds. The ammonia was allowed to evaporate and the residue dried.

The residue was applied to a Sephadex SPC 25 column (77 x 1.6 cms) eluted with 30% acetic acid at 20 mls/hr with a sodium chloride gradient 0.01 M to 1M over 2 days collecting 6.6 ml fractions.

The product was contained in fractions 88-92. These were pooled, evaporated and the residue dissolved in glacial acetic acid and filtered to remove the sodium chloride. The solution was evaporated and desalted on a Sephadex G 25 column (72 x 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6ml fractions. Fractions 32 - 41 were pooled, evaporated, transferred to a vial and lyophilised.

Yield 46.8 mg

Difference in yields between "L - His"and "D-His" compounds was account d for by a lower incorporation of the isostere in the "L" case and less removal of the histidine with Na/NH_3.

Product $C_{52}H_{74}O_9N_{12}$ MW 1011.25

Tlc (silica Rf 0.18 EtOAc/Pyr/AcOH/H_2O 40:20:6:11

Tle pH 2.1 1000V 30 min mobility 7.7 cm

pH 6.5 1000V 30 min mobility 7.9 cm

AAA 6N-HCl + phenol, 110°, 40 hours, peptide content 93%

His: 1.98; Pro:1.00; Val:1.08; Tyr: 0.97; Phe 0.97

EXAMPLE V

H-His-Pro-Phe-His-Leu-reduced-Val-Ile-His-OH (H 113)

The method is generally that of Example I above but illustrates formula (VB).

Preparation of Boc-His (DNP)-O-Resin (AH/30/83)

Boc-His(DNP)-OH. (4.74g, 11.25 mmol) was dissolved in ethanol (60ml) and a solution of cesium bicarbonate (2.18g, 11.25mmol) in water (15 ml) added. The solvent was evaporated in vacuo and the residue treated four times with toluene and evaporated to remove water before finally drying overnight over phosphorus pentoxide. The residue was dissolved in DMF (175 ml), chloromethylated resin (30g, 22.5 m-equiv.) added and the reaction stirred at 37° for five days.

The resin was filtered off and washed thoroughly with DMF, DMF/water (9:1) and then DMF again. It was resuspended in DMF (175 ml) and treated with acetic anhydride (7.08 ml, 75 mmol) and triethylamine (10.5ml, 75 mmol) overnight.

The resin ester was filtered, washed thoroughly with DMF, DMF/water (9:1) and methanol and dried. It was then"de-fined" by shaking it in dichloromethane and removing the particles in the supernatant. Finally, the resin was dried. Yield:30.45g. A trial coupling with Boc-Ala-OH, followed by amino-acid analysis (after hydrolysis with 12N-HCl/propionic acid 1:1, 130°, 2hrs) gave an incorporation of 0.22mmol/ .

Coupling to Resin Ester

Boc-His(DNP)-O-Resin (2.5g 0.55 mmol) was deprotected with 50% TFA/CH_2Cl_2 and Boc-Ile-OH (0.748g 3mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6.0mmol) for 2 hours, then acetylated with acetyl imidazole (0.55g, 5mmol) overnight*.

After deprotection with 40% TFA/CH_2Cl_2, Boc-Leu-reduced (3,4-Cl_2-Z)-Val-OH, 4 (0.343g, 0.66 mmol) was coupled using DCCI (0.15g, 0.73mmol) and HOBt (0.202g, 1.32 mmol) for 16 hours, then acetylated for 1 hour.

After deprotection, Boc-His(DNP)-OH (1.26g, 3.0 mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6mmol) for 2 hours, then acetylated for 1 hour.

After deprotection, again with 50% TFA/CH_2Cl_2, Boc-Phe-OH (0.796g, 3mmol) was coupled with DCCI (0.68g, 3.3mmol) and HOBt (0.92g, 6mmol) for 3 hours• then acetylated overnight•

After deprotection, Boc-Pro-OH (0.646g, 3mmol) was coupled using DCCI (0.68g, 3.3mmol) and HOBt (0.92g, 6mmol) for 2 hours then acetylated for 1 hour.

The peptide was again deprotected and coupled with Boc-His (DNP)-OH (1.26g, 3mmol) using DCCI (0.68g, 3.3mmol) and HOBt (0.92g, 6mmol) for 2 hours, then acetylated overnight•.

The peptide resin ester was washed with DMF (3X), CH_2Cl_2 (3X) iProH (2X), CH_2Cl_2 (3X) and finally MeOH (3X) and dried. It was then treated with HF at 0° for 1¼ hours in the presence of anisole (4ml) and dried overnight over potassium hydroxide. The resin was washed with DMF, acetic acid and acetic acid/water (1:1) to remove the peptide. The washes were combined and evaporated in vacuo.

The residue was dissolved in DMF(60 ml) and water (24ml), thioethanol (10ml) was added and the pH of the solution brought to 8.0 with sodium carbonate solution. The reaction mixture was stirred overnight, the solvent evaporated and the residue applied to a Sephadex G25 column (77 x 2.5cms). It was eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 34-53 were combined and the solvent evaporated in vacuo and dried.

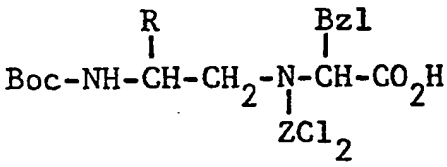
Product $C_{49}H_{72}O_3N_{14}$ MW 985,21

Tlc (silica) Rf = 0.63 in EtOAc-Py-AcOH-H_2O (15:20:6:11)

AA analysis in accordance with calculated composition.

EXAMPLES VI - IX

These Examples illustrate formula (VA). The methods disclosed above are applied to condensing Boc-Phe-H or Boc-Leu-H with H-Phe-OBzl, reducing the imine link, deprotecting at the carboxyl terminus and protecting the nitrogen of the reduced peptide link to give:



12 R = Bzl

12a R = iBu ZCl_2 = 3, 4-dichloro benzyloxy carbonyl

This Phe-reduced-Phe or Leu-reduced-Phe analogue is then used as follows:

- VI Use of 12 (Phe-reduced-Phe) in an analogue otherwise as H-77 (see Example IV)

- VII Use of 12a (Leu-reduced-Phe) in an analogue otherwise as H-77 (see Example IV)

- VIII Use of 12 in an analogue as H-76 (Example I), viz:

H-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-OH (H 110)
6 7 8 9 10 11 12 13

- IX Use of 12a in an analogue as H-76 (Example I), viz:

H-His-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH (H 115)

EXAMPLE X

This Example illustrates formula (VB), the method of Example V being used but with the Tyr resin of Examples I to IV, to give:

H-His-Pro-Phe-His-Leu-reduced-Val-Ile-Tyr-OH (H 116)
6 7 8 9 10 11 12 13

EXAMPLE XI

Use of the methods disclosed herein to give:

H-Pro-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-Lys-OH (H 108)
5 6 7 8 9 10 11 12 13 14

this being an analogue illustrating the non-criticality of the terminal portions of the chain allowing X and W in formulae (V), (VA) and (VB) to represent further residues. It is a further example of formula (VA).

EXAMPLE XII

Use of the methods disclosed herein to give:

H-His-Pro-Phe-His-Leu-reduced-Val-Val-Tyr-OH (H 117)
6 7 8 9 10 11 12 13

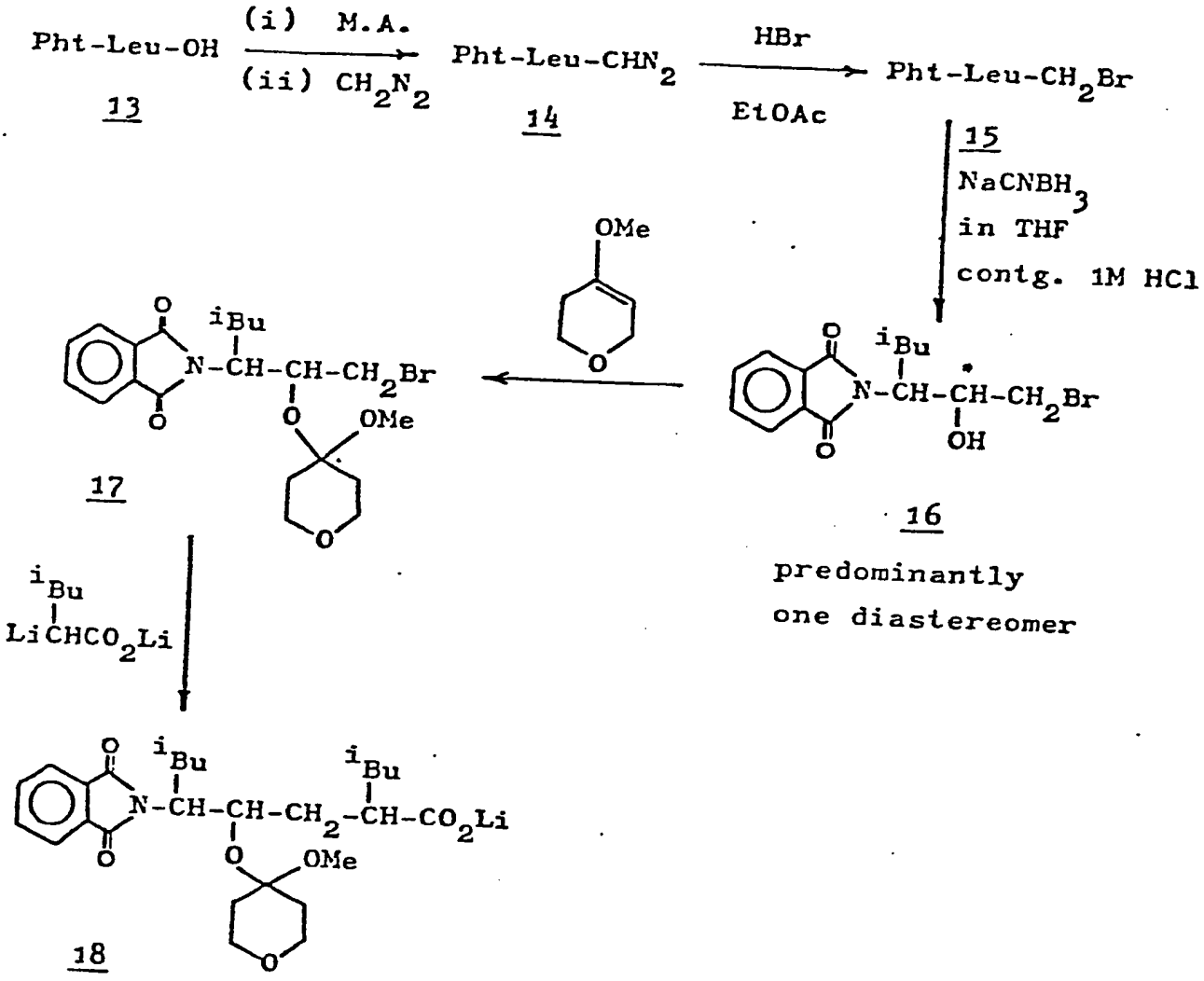
a compound of value in its relation to the Leu-Val structure at 11, 12 in human renin substrate.

The following Example illustrates the synthesis of hydroxy and keto isosteres.

EXAMPLE XIII

The reaction scheme below, also suitable for other hydroxy dipeptide analogues, was used to synthesise an N-terminal and hydroxy-group protected Leu-Leu hydroxy isostere 18.

Scheme 4



M.A. = isobutyl chloroformate/triethylamine Pht = phthaloyl

The resulting N-terminal phthaloyl protected, -OH protected hydroxy isostere of Leu-Leu can be coupled direct for example to valyl tyrosine, followed by removal of the phthaloyl group, coupling direct to a suitable tri or tetrapeptide, and deprotection at the -OH group by mild acid hydrolysis, to give for example analogues corresponding to H-76 (Example 1), H-79 (Example 2), H-77 (Example 4). Alternatively the phthaloyl group may be removed by treatment with hydrazine and a new protective group, e.g. benzyloxycarbonyl or t-butyloxycarbonyl attached prior to coupling.

The methods used after the preparation of the protected hydroxy isostere are those of the peptide synthesis art, well known in themselves and exemplified in detail herein. The compounds specifically prepared are:

a) H-His-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH

b) H-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH

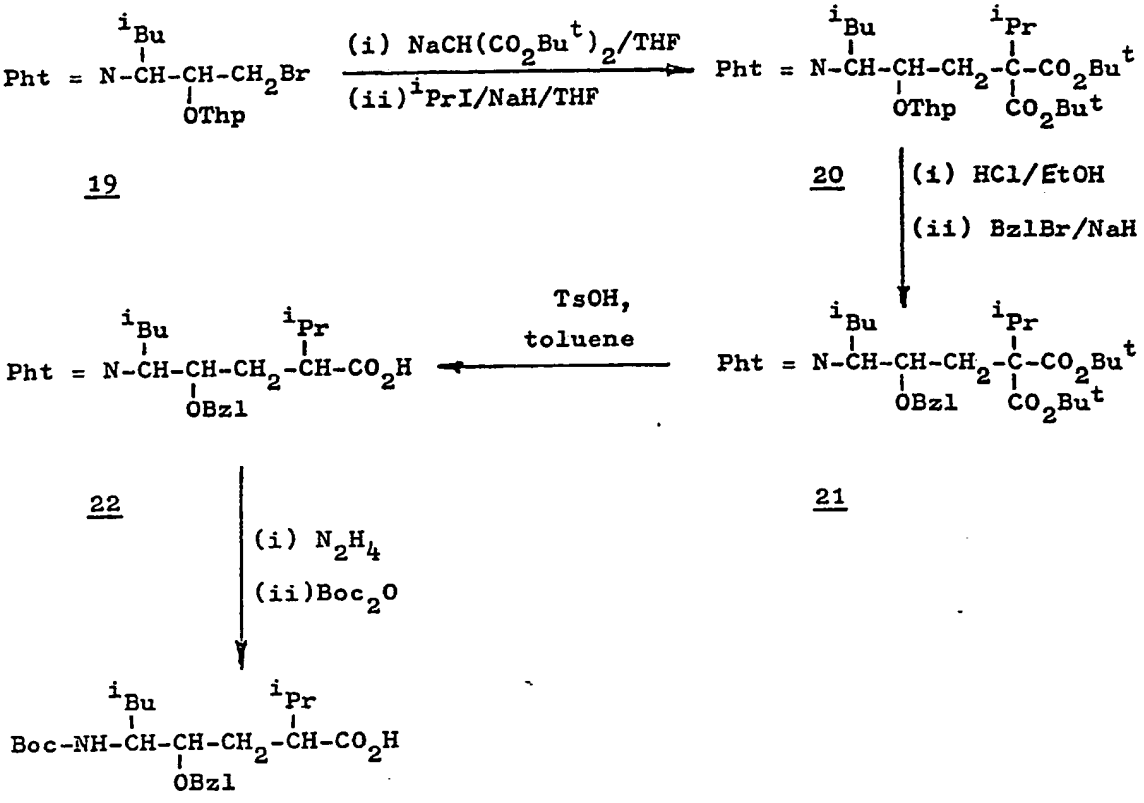
c) H-DHis-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH

EXAMPLE XIV

The alternative and preferred reaction scheme below, also suitable for other dipeptide hydroxy isosteres was used to synthesise an N-terminal and hydroxy-group protected Leu-Val hydroxy isostere 23.

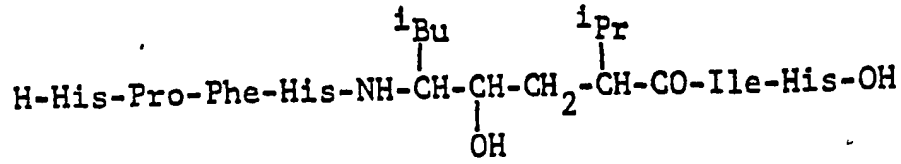
Scheme 5

Pht = Phthaloyl. Thp = Tetrahydropyranyl



In the above scheme the protected bromohydrin 19 is obtained in the same way as the corresponding intermediate 17 in Scheme 4, and is subjected to malonic ester synthesis and alkylation with isopropyl iodide to give the malonic ester derivative 20. Protection on the hydroxyl function is changed from Thp to Bzl to yield 21 and the latter is subjected to protonolysis and decarboxylation. In the resulting isostere acid 22 amino protection is changed from Pht to Boc yielding the protected isostere 23 which is suitable for incorporation into an octapeptide analogue by the usual methods of solid phase peptide synthesis to

give for example the octapeptide analogue:

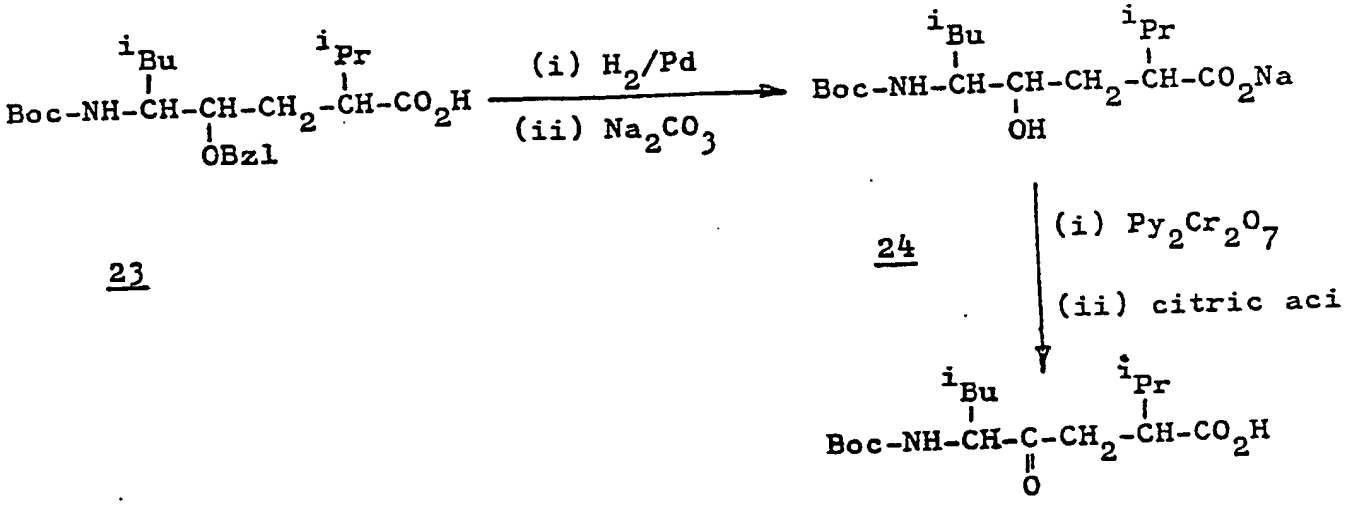


This is a Leu-hydroxy-Val isostere.

EXAMPLE XV

Keto isosteres may be prepared for example by the method of published U.K. Specification No. 1 587 809 (U.S. 4 242 256) of R. Sharpe and one of the present inventors M. Szelke, to which reference may be made. Alternatively they may be prepared from hydroxy isosteres prepared as disclosed herein and in particular, in the present Example, from the final product 23 of scheme 5 as in the scheme below:-

Scheme 6



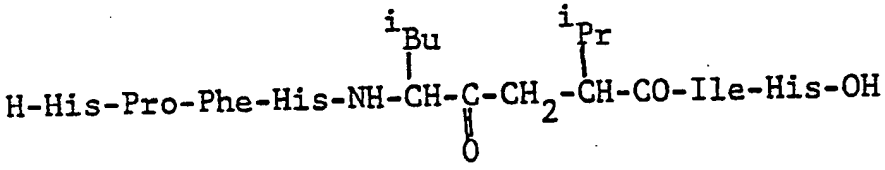
23

24

25

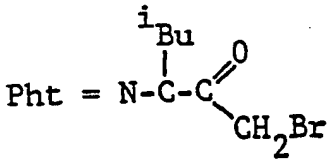
In the above scheme the benzyl protection is selectively removed from the hydroxyl function of 23 and the free acid is is converted into its sodium salt 24. The latter is subjected

to oxidation by pyridinium dichromate and acidification to give the partially protected keto isostere acid which is ready for incorporation into an octapeptide analogue by the usual methods of solid phase synthesis, for example the analogue:



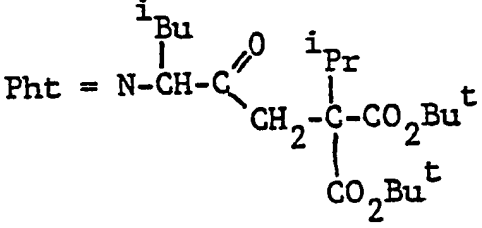
This is a Leu-keto-Val isostere.

Alternatively the keto isostere may be prepared directly by a modified version of scheme 5 wherein the bromohydrin 19 is replaced by the bromoketone 15 (scheme 4), which is:



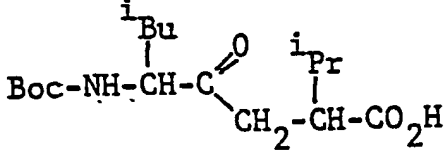
15

giving the compound



26

which is successively treated with TsOH, toluene and i) N_2H_4, ii) Boc_2O to give:



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ACTIVITY IN VITRO

Preliminary activity test results in the human renin-renin substrate reaction in vitro are given in the table below, with comparative figures for the tetrapeptide analogue (III). The test is based on the methods described by J.A. Millar et al. in Clinica Chimica Acta (1980) 101 5-15 and K. Poulsen and J. Jorgensen in J. Clin. Endocrinol. Metab. (1974) 39 816.

It is based on the measurement, by radioimmunoassay, of Angiotensin-I released from human renin substrate by human renin in human plasma. The inhibitor is dissolved in 0.01 N HCl (10 µl) and added to human plasma (75 µl) containing EDTA, and angiotensin-I antibody (15 µl) in 3M-Tris/HCl buffer (pH 6.9).

After incubation at 37°C for 0-120 mins., the enzymic reaction is quenched by the addition of ice-cold 0.25M Tris/HCl buffer (pH 7.4) containing 0.01% of bovine serum albumin. 125I-labelled angiotensin-I is added, followed by equilibration at 4°C for 48 hours. Free and bound ligand are separated by the addition of dextran-coated charcoal, and the amount of bound radio-ligand determined in a gamma counter.

The results for the renin inhibitory activities of the present compounds thus tested, expressed as the IC_{50} (the molar concentration required to cause 50% inhibition), are as follows:

Analogue		IC_{50}	
Example I	(H-76)	1.0 µM	
Example II	(H-79)	17.0 µM	
Example III	(H-78)	1.5 µM	
Example IV	(H-77)	1.0 µM	
Example V	(H-113)	0.26 µM	**
Example VI			*
Example VII			*
Example VIII	(H-110)	1.3 µM	
Example IX	(H-115)	2.5 µM	
Example X	(H-116)	0.20 µM	
Example XI	(H-108)	0.05 µM	
Example XII	(H-117)		*
Example XIII	a)		*
	b)		*
	c)		*
Example XIV			*
Example XV			*

* Preliminary indication of comparable activities.

** The corresponding non-isosteric peptide has for example been tested and shows a potency over three orders of magnitude less (IC_{50} 400 µM)

(Comparative) III 822 µM

These are most notable results, showing a potency, in the reduction of renin activity remaining in the plasma in the presence of the analogue, several orders of magnitude greater than the previously proposed tetrapeptide analogue.

ACTIVITY IN VIVO

The activity tests below are in animals but indicate corresponding activity in man.

In in vitro studies, in plasma from both normal and sodium-depleted dogs, the compound H-77 (Example IV) inhibited renin. In in vivo studies H-77 was infused into normal conscious sodium-depleted dogs at rates of 0.01, 0.1, 1 and 10 mg/kg/hr. A maximum fall in blood pressure, plasma renin (PR) angiotensin-I (A-I) and angiotensin-II (A-II) levels was obtained within 10 minutes at doses of 1 and 10 mg/kg/hr. When the infusion was stopped, blood pressure returned to baseline levels 30 minutes after the 1 mg/kg/hr. dose, but more slowly after the 10 mg/kg/hr. dose.

In the claims below it will be understood that compounds may be in the form shown or in protected or salt form at NH_2, COOH, OH or other active groups and in particular as their physiologically acceptable acid addition salts at basic centres. Further as already noted herein general reference to amino acids and amino acyl residues and side chains is to be taken as reference to such whether naturally occurring in proteins or not and to both D- and L- forms, and amino is to be taken as including imino except where an aromatic acid, residue or side chain is specified.

CLAIMS:

1. A polypeptide analogue of the formula:

X-Y-Pro-Phe-His——A——B—Z—W (V)

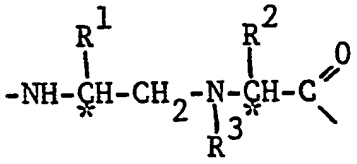
6 7 8 9 10,11 12 13

where:-

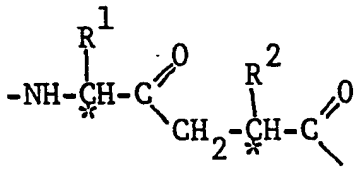
Pro, Phe and His may be in substituted form;

X = H; or an acyl or other N-protecting group e.g. acetyl, pivaloyl, t-butyloxycarbonyl (Boc), benzoyl or lower alkyl (primarily C_1-C_5); or an L- or D-amino- acyl residue, which may itself be N-protected similarly;

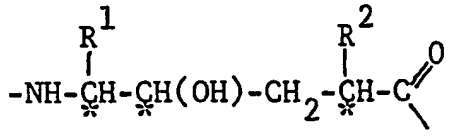
Y = D- or L-His or other D- or L- basic or aromatic amino-acyl residue, or is absent;

A =  (VI) "reduced" isostere bond

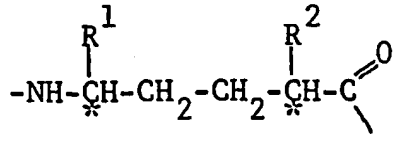
or

 (VII) "keto" isostere bond

or

 (VIII) "hydroxy" isostere bond

or

 (IX) "hydrocarbon" isostere bond

where the configuration at asymmetric centres * is either

R or S, where in VIII the hydroxy group may be present as such or protected in ether $-OR^4$ or ester $-O-C(=O)-R^4$ form

where R^4 is as given under W below and where

R^1 and R^2, the same or different = ^{i}Pro (isopropyl), ^{i}Bu (isobutyl), Bzl (benzyl) or other lipophilic or aromatic amino-acid side chain

R^3 = -H; lower alkyl (C_1-C_5); or $-SO_2Ph$, $-SO_2C_6H_4CH_3$(p), Boc, formyl or other N-protecting group;

B = D- or L- Val or Ile or other D- or L- lipophilic amino-acyl residue;

Z = D- or L- Tyr, Phe, His or other L- or D-aromatic amino-acyl residue;

and

W = -OH as such or in protected ester form as $-OR^4$ where R^4 = lower alkyl primarily C_1-C_5 and particularly ^{t}Bu, or cycloalkyl primarily C_3-C_7, or Bzl, or other ester forming group; or $-NH_2$ as such or in protected amide form as $-NHR^5$ or $-N(R^5)_2$ (where R^5 = an N-protecting or other substituent group e.g. lower alkyl as for R^4 and $(R^5)_2$ = two such or e.g. cycloalkyl, primarily C_3-C_7) or as $-NH-(CH_2)_n-Q$ or $-NR^5-(CH_2)_n-Q$ (where n = 2 to 6 and Q = NH_2 or $-NH-C(=NH)-NH_2$ and wherein any of the hydrogens attached to nitrogen may be substituted by R^5 or $(R^5)_2$); an L- or D-

serine or lysine, arginine or other basic amino-acyl residue as such or in amide form, substituted amide form or ester form e.g. containing a group or groups as given for R^4 and R^5 above as the case may be; or an amino alcohol residue derived therefrom as such or protected in ester or ether form e.g. containing a group as given for R^4 above

or

Z + W = an alcohol derived from L- or D-Tyr, Phe, His or other L- or D- aromatic amino-acyl residue as such or protected in ester or ether form as above;

such polypeptide being in the above form or modified by isosteric replacement of one or more remaining peptide bonds by reduced, $-CH_2-NH-$, keto, $-C(=O)-CH_2-$, hydroxy, $-CH(OH)-CH_2-$, or hydrocarbon, $-CH_2-CH_2-$ isosteric links and further being in free form or in protected form at one or more remaining peptide, carboxyl, amino, hydroxy or other reactive groups.

2. A polypeptide analogue, according to claim 1, of the formula:

X-Y-Pro-Phe-His——A——Val—Z—W (VA)

6 7 8 9 10,11 12 13

where

X, Y, Pro, Phe and His are as in claim 1

A is as in claim 1 except that

R^1 and R^2, the same or different = ^iBu (isobutyl) or Bzl (benzyl) or other lipophilic or aromatic amino-acid side chain

R^3 = -H; or $-SO_2Ph$, $-SO_2C_6H_4CH_3(p)$, Boc, formyl or other N-protecting group

Z = Tyr, Phe or other L- or D-aromatic amino-acyl residue;

W = -OH as such or in protected ester form as $-OR^4$ where R^4 = lower alkyl (primarily C_1-C_5 and particularly ^tBu), or Bzl, or other ester forming group ; or $-NH_2$ as such or in protected amide form as $-NHR^5$ or $-N(R^5)_2$ (R^5 = an N-protecting group e.g. lower alkyl as for R^4; $(R^5)_2$ = two such or e.g. cyclo-alkyl, primarily C_3-C_7) or

an L- or D- amino-acyl residue e.g. a serine or basic amino-acyl residue as such or in amide form or in protected amide or ester form e.g. containing a group or groups as given for R^4 and R^5 above as the case may be; or an amino acid alcohol residue derived therefrom as such or protected in ester or ether form e.g. containing a group as given for R^4 above

or

Z + W = an alcohol derived from Tyr or Phe or other L- or D- aromatic amino acyl residue as such or protected in ester or ether form as above.

3. A polypeptide analogue, according to claim 1, of the formula:

X-Y-Pro-Phe-His——A——Ile—Z—W (VB)

6 7 8 9 10,11 12 13

where

X, Y, Pro, Phe and His are as in claim 1

A is as in claim 1 except that

R^1 = ^{i}Bu (isobutyl) or Bzl (benzyl) or other lipophilic or aromatic amino-acid side chain

R^2 = ^{i}Pr (isopropyl), and

R^3 = -H; or $-SO_2Ph$, $-SO_2C_6H_4CH_3(p)$, Boc, formyl or other N-protecting group

Z is as in claim 1

W is as in claim 2

or Z + W = an alcohol derived from the aromatic residues specified for Z in claim 1, as such or protected in ester or ether form as specified therein.

4. A polypeptide according to any one of claims 1 to 3, modified by isosteric replacement, as set out therein, at one or both of the Pro-Phe or Phe-His links.

5. A polypeptide according to any one of claims 1 to 4 wherein the isosteric replacement at at least the 10,11 position is of the "reduced" kind.

6. A polypeptide according to any one of claims 1 to 4 wherein the isosteric replacement at at least the 10,11 position is of the "hydroxy" kind.

7. The compound:

H-His-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH.

8. The compound:

H-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH.

9. The compound:

H-His-Pro-Phe-His-Leu-reduced (SO_2Ph)-Leu-Val-Tyr-OH.

10. The compound:

H-DHis-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH.

11. The compound:

H-His-Pro-Phe-His-Leu-reduced-Val-Ile-His-OH.

12. The compound:

H-DHis-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-OH.

13. The compound:

H-DHis-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH.

14. The compound:

H-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-OH.

15. The compound:

H-His-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH.

16. The compound:

H-His-Pro-Phe-His-Leu-reduced-Val-Ile-Tyr-OH.

17. The compound:

H-Pro-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-Lys-OH.

18. The compound:

H-His-Pro-Phe-His-Leu-reduced-Val-Val-Tyr-OH.

19. The compound:

H-His-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH.

20. The compound:

H-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH.

21. The compound:

H-DHis-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH.

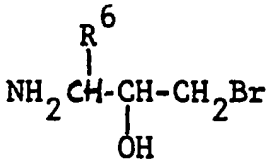
22. The compound:

H-His-Pro-Phe-His-Leu-hydroxy-Val-Ile-His-OH.

23. The compound:

H-His-Pro-Phe-His-Leu-keto-Val-Ile-His-OH.

24. A method of making a hydroxy or keto isostere of a dipeptide wherein a derivative of a halohydrin preferably a bromohydrin or haloketone preferably a bromoketone



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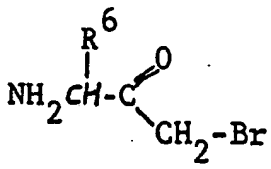
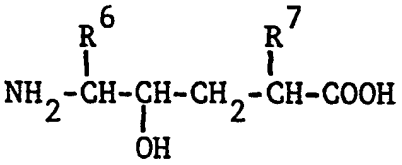
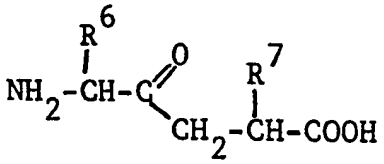


wherein R^6 is an amino acid side chain and the NH_2 and OH groups are in protected form is alkylated to give the desired isostere

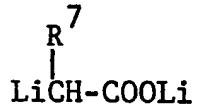


or



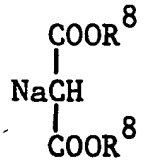
as such or in protected form, R^7 being the same or a different amino acid side chain.

25. A method according to claim 24 wherein the alkylation procedure is by reaction with an alkali metal carboxylic acid derivative preferably a lithium derivative



where R^7 is as in claim 24.

26. A method according to claim 25 wherein the alkylation procedure is by reaction with an alkali metal malonic ester derivative preferably a sodium derivative

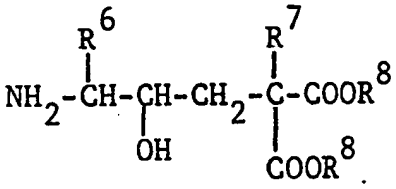


where R^8 is an esterifying group and a halide preferably an

iodide

$$R^7\text{-}I$$

where R^7 is as in claim 24 to give intermediate



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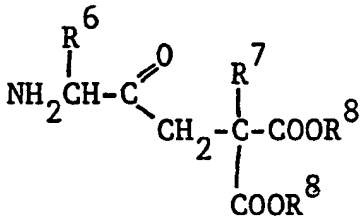
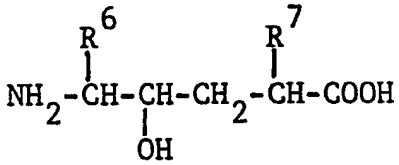


in protected form which intermediate is then decarboxylated and if desired deprotected to give the desired isostere



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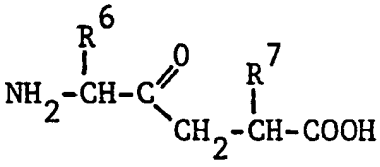
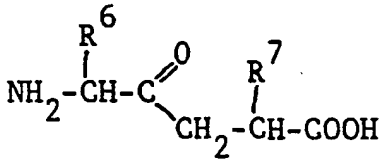


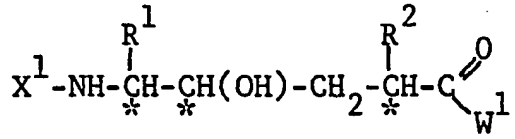
as such or in protected form.

27. A method of making a keto isostere of a dipeptide wherein a hydroxy isostere is made by the method of any of claims 24 to 26 and the -CH(OH)- group is oxidised to give the desired isostere



as such or in protected form.

28. The method of any of claims 24 to 27 when applied to the production of a hydroxy dipeptide isostere of the formula



or the corresponding keto isostere , where the significance of *, X^1 and W^1 is as in claim 1 except that X^1 and W^1 do not represent amino-acyl.

29. A hydroxy or keto isostere of a dipeptide when made by the method of any of claims 24 to 28, as such or in the form of a higher polypeptide analogue made using it.

30. In a diagnostic test for high-renin states, blood pressure falling most when renin is high, and as a surgical prognostic test for reno-vascular hypertension (renal artery stenosis), the administration of a polypeptide analogue according to any one of claims 1 to 23 followed by monitoring of blood pressure, and such polypeptide analogues when for such use.

31. In the long and short term treatment of heart failure and all forms of hypertension particularly those associated with high serum renin levels, the administration of a renin-inhibiting amount of a polypeptide analogue according to any one of claims 1 to 23, and such polypeptide analogues when for such use.

32. Administration according to claim 30 or 31, of 0.001 to 10 mg/kg body weight daily, preferably 0.01 to 1.0 mg, of the polypeptide analogue.

33. A polypeptide analogue according to any one of claims 1 to 23, when in the form of a composition with a pharmaceutically acceptable diluent or carrier.

34. The composition of claim 33, in unit dosage form containing the amounts of analogue set out in claim 32 or sub-multiple thereof.

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European Patent Office

PARTIAL EUROPEAN SEARCH REPORT
which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application number
EP 81 30 3585.4

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim
D	US - A - 4 198 398 (HUDSON et al.) * column 4, line 64 to column 7, line 45 * --	24,31, 33
	DE - A1 - 2 714 141 (NATIONAL RESEARCH DEVELOPMENT CORP.) * claims; examples 1 to 6 * --	24,31, 33
	DE - A1 - 2 811 267 (NATIONAL RESEARCH DEVELOPMENT CORP.) * claims; example * --	24,31, 33
A	Chemical Abstracts vol. 75, no. 23, December 1971 Columbus, Ohio, USA K. SHIGEZANE et al. "Synthesis of anti-renin active peptides. II." page 350, abstract no. 141149r & Yakugaku Zasshi, vol. 91, no. 9, 1971, pages 987 to 996 -- ./..	1-23

CLASSIFICATION OF THE APPLICATION (Int. Cl.³)

C 07 C 103/52
C 07 C 101/30
C 07 C 101/34
A 61 K 37/02
//C 07 C 101/04
C 07 C 101/24

TECHNICAL FIELDS SEARCHED (Int.Cl.³)

C 07 C 101/30
C 07 C 101/34
C 07 C 103/52

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely: 1-31, 33
Claims searched incompletely: --
Claims not searched: 32, 34
Reason for the limitation of the search:

Method for treatment of the animal or human body by therapy

CATEGORY OF CITED DOCUMENTS

X: particularly relevant
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention
E: conflicting application
D: document cited in the application
L: citation for other reasons

&: member of the same patent family, corresponding document

Place of search	Date of completion of the search	Examiner
Berlin	05-10-1981	BREW

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application number EP 81 30 3585.4

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.³)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
	Chemical Abstracts vol. 85, no. 17 October 1976 Columbus, Ohio, USA M.J. PARRY et al. "Bio-isosteres of a peptide renin inhibitor" page 65, abstract no. 117050n & Chem. Biol. Pept., Proc. Am. Pept. Symp. 3rd , 1972, pages 541 to 544 —	1-31, 33	
A	Chemical Abstracts vol. 80, no. 1, January 1974 Columbus, Ohio, USA I. PARIKH et al. "Substrate analog competitive inhibitors of human renin" page 108, abstract no. 1086w & Biochem. Biophys.Res. Commun.,vol. 54, no. 4, 1973, pages 1356 to 1361 —	1-23	TECHNICAL FIELDS SEARCHED (Int. Cl.³)
A	Chemical Abstracts vol. 87, no. 8, July 1977 Columbus, Ohio, USA K. POULSEN et al. "Competitive inhibitors of renin. A review." page 139, abstract no. 1561s & Prog. Biochem. Pharmacol., vol. 12, 1976, pages 135 to 141 —	1-23	

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(54) **Enzyme inhibitors.**

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(56) References cited:
DE-A-2 714 141
DE-A-2 811 267
US-A-4 198 398

Chemical Abstracts vol. 75, no. 23, December 1971 Columbus, Ohio, USA K. SHIGEZANE et al. "Synthesis of antirenin active peptides. II." page 350, abstract no. 141149r

Chemical Abstracts vol. 85, no. 17 October 1976 Columbus, Ohio, USA M.J. PARRY et al. "Bio.isosteres of a peptide renin inhibitor" page 65, abstract no. 117050n

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(56) References cited:
Chemical Abstracts vol. 80, no. 1, January 1974 Columbus, Ohio, USA I. PARIKH et al. "Substrate analog competitive inhibitors of human renin" page 108, abstract no. 1086w

Chemical Abstracts vol. 87, no. 8, July 1977 Columbus, Ohio, USA K. POULSEN et al. "Competitive inhibitors of renin. A review" page 139, abstract no. 1561s

Courier Press, Leamington Spa, England.

EP 0 045 665 B1

Description

The invention relates to renin-inhibiting peptide analogues.

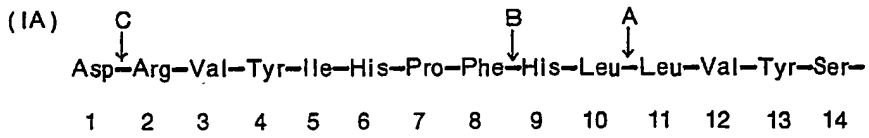
Background

Renin is a natural enzyme, disorders in relation to which are implicated in many cases of hypertension. It is released into the blood from the kidney, and cleaves from a blood glycoprotein a decapeptide known as angiotensin-I. Circulating angiotensin-I is cleaved in lung, kidney and other tissues to an octapeptide, angiotensin-II, which raises blood pressure both directly by causing arteriolar constriction and indirectly by stimulating release of the sodium-retaining hormone aldo-sterone from the adrenal gland and thus causing a rise in extracellular fluid volume. The latter effect is caused by angiotensin-II itself or a heptapeptide cleavage product angiotensin-III.

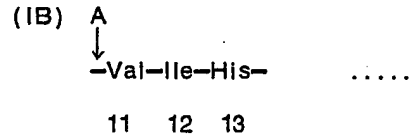
Inhibitors of renin have therefore been sought, with two ends in view, first the provision of a diagnostic agent for identification of cases of hypertension due to renin excess, and secondly the provision of an agent for control of hypertension in such cases.

The present inventors' approach has been to consider the peptide sequence characterising the natural renin substrate at its binding site, and to seek peptide analogues sufficiently similar to bind to the enzyme, in competition with the natural substrate, but sufficiently dissimilar to it to be cleaved slowly or not at all. Such analogues will block the action of the enzyme and attack the hypertension at source.

Renin is specific to a particular bond in the substrate, the N-terminal sequence of which in the horse is for example:

(IA) 

as found by L. T. Skeggs et al J. Exper. Med. *106* 439 (1957). Human renin substrate has a different sequence recently discovered by D. A. Tewkesbury et al Biochem. Biophys. Res. Comm. *99* 1311 (1981)

(IB) 

the sequence to the left of the arrow A being as in formula (IA).

Cleavage at A gives angiotensin-I; subsequent cleavage at the Phe-His bond at B gives angiotensin-II; and cleavage subsequently again at the Asp-Arg bond at C gives angiotensin-III.

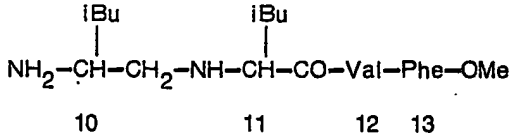
Peptides similar to certain partial sequences of the substrate have been shown to act as inhibitors of renin in vitro. An example is the tetrapeptide ester (the relation to the substrate residues being indicated by numbering):

(II) H-Leu-Leu-Val-Phe-OMe

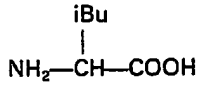
10 11 12 13

proposed by Kokubu, Nature, *217* 456 (1968) but it is inactive in vivo, because of binding to plasma proteins and rapid attack by natural peptidases.

One of the present inventors undertook some years ago a development of Kokubu's work, seeking a renin inhibitor active in vivo, in which analogues of peptides similar to Kokubu's were made by having a methylene imino group —CH_2—NH— in place of the peptide link —CO—NH— between the leucine residues. One of these analogues was:

(III) 

which is the tetrapeptide (I) modified at the Leu-Leu link, leucine of course being



(IV)

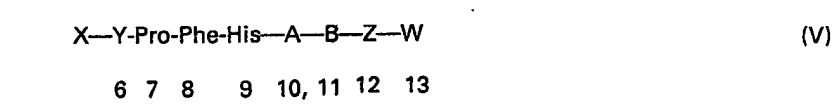
This analogue (III) was the first effective in-vivo inhibitor of renin and was shown to have significant anti-hypertensive action in Goldblatt hypertensive rats (Parry, Russell and Szelke p. 541 in "Chemistry and Biology of Peptides" Ed. Meienhofer, Ann Arbor Science Publishers 1972). Little or no attention has however been paid to the work, which the authors themselves were unable to pursue, in spite of considerable activity in the general field of substrate-based inihibitors for renin, reviewed for example by Haber & Burton, Federation Proc. *38* No. 13 2768—2773 (1979).

The Invention

The present invention is a development of the above work. Behind it is a concept of modifying peptide structures related to the peptide sequence at the site of action of renin on the natural substrate, by isosteric substitution at, at least, the site of cleavage. Optionally further there is isosteric substitution or other modification at other positions to increase stability or to modify the properties of the final peptide, for example its solubility under physiological conditions or its resistance to in vivo exopeptidase attack. Such modification may for example be by incorporation of residues other than those of the natural L-amino acids; by protection of the N-terminus with acetyl, pivaloyl, t-butyloxycarbonyl (Boc), benzoyl or other groups; or by conversion of the C-terminal carboxyl to another functional group, e.g. the corresponding alcohol, present as such or in ether or ester form.

General reference to amino acids and amino acyl residues and side chains in both the description and claims herein is to be taken as reference to such whether naturally occurring in proteins or not and to both D- and L- forms, and amino is to be taken as including imino except where an aromatic acid, residue or side chain is specified.

The compounds of the present invention, showing desirable renin inhibitory action, are of the general formula:

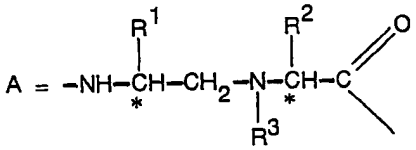


(V)

where Pro, Phe and His may be in substituted form;

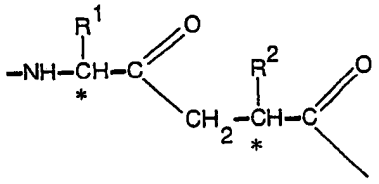
X = H; or an acyl or other N-protecting group e.g. acetyl, pivaloyl, *t*-butyloxycarbonyl (Boc), benzoyl or lower alkyl (primarily C_1—C_5); or an L- or D-amino- acyl residue, which may itself be N-protected similarly;

Y = D- or L-His or other D- or L- basic or aromatic amino-acyl residue, or is absent;



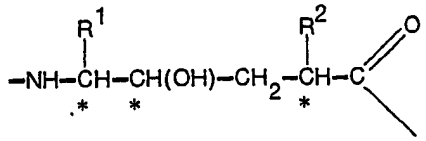
(VI) "reduced" isostere bond

or

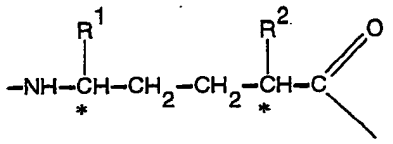


(VII) "keto" isostere bond

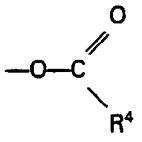
or



(VIII) "hydroxy" isostere bond

or  (IX) "hydrocarbon" isostere bond

where the configuration at asymmetric centres * is either R or S, where in VIII the hydroxy group may be present as such or protected in ether —OR^4 or ester



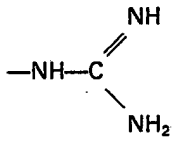
form where R^4 is as given under W below and where R^1 and R^2, the same or different = ^{i}Pro (isopropyl), ^{i}Bu (isobutyl), Bzl (benzyl) or other lipophilic or aromatic amino-acid side chain

R^3 = —H; lower alkyl (C_1—C_5); or —SO_2Ph, —$SO_2C_6H_4CH_3$(p), Boc; formyl or other N-protecting group;

B=D- or L- Val or Ile other D- or L- lipophilic aminoacyl residue;

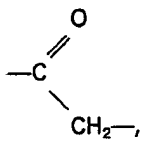
Z = D- or L- Tyr, Phe, His or other L- or D- aromatic aminoacyl residue; and

W = —OH as such or in protected ester form as —OR^4 where R^4 = lower alkyl primarily C_1—C_6 and particularly ^{t}Bu, or cycloalkyl primarily C_3—C_7, or Bzl, or other ester forming group; or —NH_2 as such or in protected amide form as —NHR^5 or —$N(R^5)_2$ (where R^5 = an N-protecting or other substituent group e.g. lower alkyl as for R^4 and $(R^5)_2$ = two such or e.g. cycloalkyl, primarily C_3—C_7) or as —NH—$(CH_2)_n$—Q or —NR^5—$(CH_2)_n$—Q (where n = 2 to 6 and Q = NH_2 or



and wherein any of the hydrogens attached to nitrogen may be substituted by R^5 or $(R^5)_2$); and L- or D- serine or lysine, arginine or other basic amino-acyl residue as such or in amide form substituted amide form or ester form e.g. containing a group or groups as given for R^4 and R^5 above as the case may be; or an amino alcohol residue derived therefrom as such or protected in ether or ether form e.g. containing a group as given for R^4 above or Z + W = an alcohol derived from L- or D- Tyr, Phe, His or other L- or D- aromatic amino-acyl residue as such or protected in ester or ether form as above;

such polypeptide being in the above form or modified by isosteric replacement of one or more remaining peptide bonds by reduced —CH_2—NH—, keto,

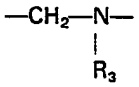


hydroxy, —CH(OH)—CH_2—, or hydrocarbon, —CH_2—CH_2— isosteric links and further being in free form or in protected or salt form at one or more remaining peptide, carboxyl, amino, hydroxy or other reactive groups, in particular as their physiologically acceptable acid addition salts at basic centres.

The above compounds may in particular be those related to the substrate sequence in the horse (B = Val at position 12) or those related to the substrate sequence in man (B = Ile at position 12). Particular groups of these compounds are set out in claims 2 and 3 respectively herein, as formulae VA and VB to which reference may be made but which are not repeated at this point.

The numbering of residues in formulae (V), (VA) and (VB) shows the correspondence with the renin substrates themselves, but without limitation of the generality of the formulae.

Where a peptide bond in addition to that corresponding to the Leu-Leu or Leu-Val bond in the natural renin substrate is isosterically substituted, the 7, 8 and 8, 9 positions i. . th Pro-Phe and Ph -His bonds in formula V are preferred, or possibly both of th se p sitions, and it is further preferred that th substitution should be

 (X)

where R_3 is as set out above. The alternative isosteric substitutions s t out herein may however be used.

Protective or substituent groupings as mentioned above may be any of these known in the polypeptide art amply disclosed in the literature and not requiring discussion at length here. Gen rally the selection of th 'protectiv ' groups is according to their function, s me being primarily int nded to prot ct against undesired reacti n during synthetic procedures whil the N- and C- terminal substituents are for example directed against the attack of exopeptidases on the final compounds or to increas their solubility and hence physiological acceptability.

It is in particular possible for one or more remaining peptide bonds in the compounds of formula (V), (VA) or (VB) to be N-substituted with protective groups.

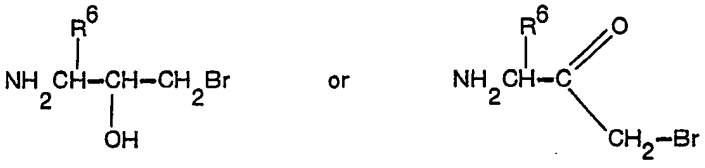
The invention further lies in the analogues for use as medicaments and particularly

i) in a diagnostic test for high renin states, blood pressure falling most when renin is high, or in a surgical prognostic test for reno-vascular hypertension (renal artery stenosis), by administration of said analogue followed by monitoring of blood pressure, and

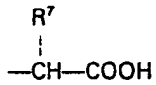
ii) in the long and short term treatment of heart failure and all forms of hypertension particularly those associated with high serum renin levels, by administration of a renin-inhibiting amount of said analogue.

The long and short term response of blood pressure to renin inhibitors is predictive of surgical outcome. In all cases single and repeated doses and any conventional form of pharmaceutical composition may be used, for administration by intranasal or oral route, injection, or any other means as convenient. Amounts may for example be 0.001 to 10 mg/kg body weight daily more usually 0.01 to 1 mg, according to the potency of the analogue and the severity of the condition. Dosage unit compositions may contain such amounts or submultiples thereof to make up the daily dose. (Dosages herein and in the claims are related to the free base content where compounds are in salt form)

In production of the analogues use may be made of a method of making a hydroxy or keto isostere of a dipeptide wherein a derivative of a halohydrin preferably a bromohydrin or haloketone preferably a bromoketone



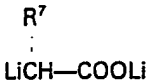
wherein R^6 is an amino acid side chain and the NH_2 and OH groups are in protected form is subjected to an alkylation procedure to attach a group



and gives the desired isostere as such or in protected form, R^7 being the same or a different amino acid side chain.

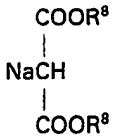
In particular the alkylation procedure may be

i) by reaction with an alkali metal carboxylic acid derivative preferably a lithium derivative

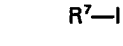


where R^7 is as above.

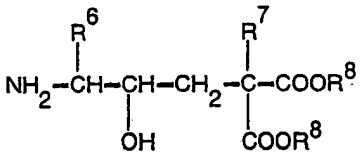
ii) by reaction with an alkali metal malonic ester derivative preferably a sodium derivative



where R^8 is an esterifying group and a halide preferably an iodide



wher R^7 is as above to give intermediat



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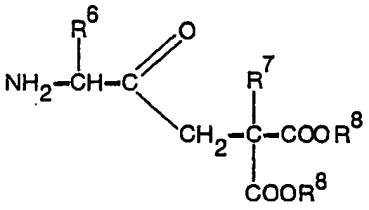
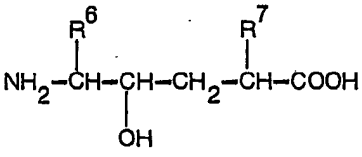
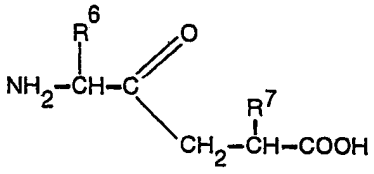


in protected form which intermediate is then decarboxylated and if desired deprotected to give the desired isostere



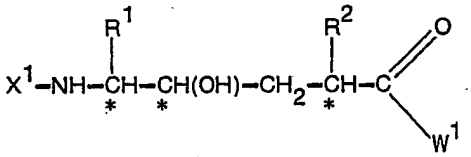
or



as such or in protected form.

The hydroxy isosteres so produced may further be oxidised to the corresponding keto isosteres.

In particular the methods may be applied to the production of a hydroxy dipeptide isostere of the formula

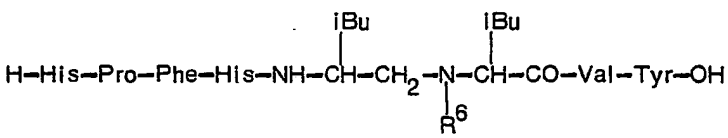


or the corresponding keto isosteres, where the significance of *, X^1 and W^1 is as above except that X^1 and W^1 do not represent amino-acyl.

The dipeptide isosteres given by all these methods may be incorporated in higher peptide analogues by the methods herein described or by the methods of peptide synthesis as generally known in the art.

The dipeptide analogue syntheses are illustrated in detail herein, in the course of illustrating the preparation of the octapeptides and related compounds to which the invention relates.

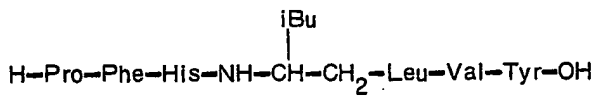
Specific analogues within the invention, all as such or in protected form, are



6 7 8 9 10 11 12 13

(H–76 where R^6 = hydrogen; H–78 where R^6 = SO_2Ph)

and the corresponding analogue (H—77) with R^6 = hydrogen and D-His at position 6. A further analogue, with the same methylene-imino isosteric replacement of a Leu-Leu peptide bond is:

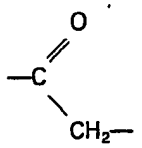


7 8 9 10 11 12 13 (H–79)

Furth r anal gues within formula (VA) ar given in the present disclosure in Exampl s VI to IX, XI and XII. Analogues within formula (VB) are given in Examples V and X.

Synthetic M th ds

The inventors have developed synth tic methods for the isosteric replacement of the peptide bond —CO—NH— with alternative groups, specifically —CH_2—NH— (reduced), —CH_2CH_2— (hydrocarbon),

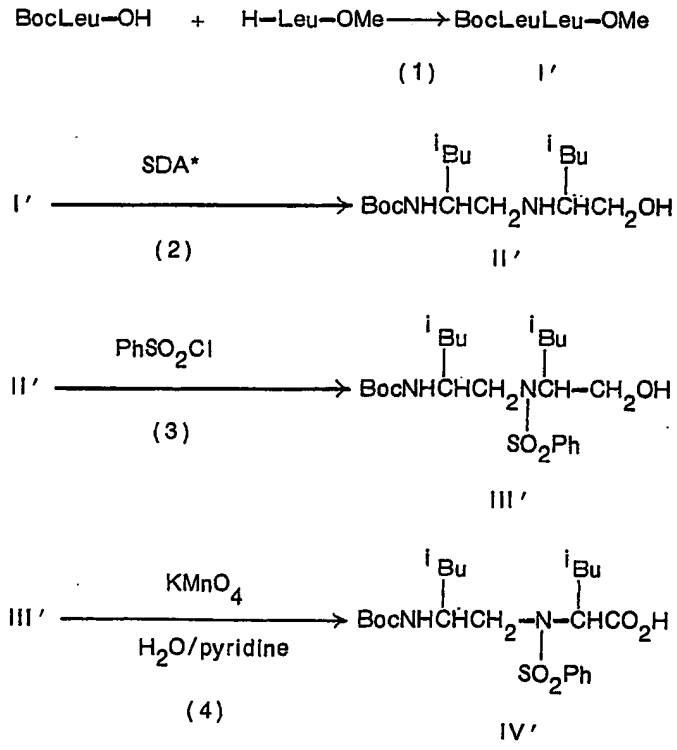


(keto) and —CH(OH)—CH_2— (hydroxy) isosteres (see, e.g. Szelke, et al, pp. 57—70 in "Molecular Endocrinology" Vol. *1*, Editors: MacIntyre and Szelke, Elsevier, Amsterdam 1977, and Hudson, Sharpe and Szelke, U.S. Patent 4 198 398 "Enkephalin Analogues").

Reference may be made to these publications for general discussion of such isosteric replacement. A reaction sequence for the preparation in particular of the reduced isostere of leucyl leucine for incorporation in the analogues disclosed herein is however for example:

SCHEME 1

Synthesis of the protected reduced isostere of L-leucyl-L-leucine



* Sodium di(methoxyethoxy) aluminium hydride

(1) Boc-Leucyl-leucine methyl ester

The dipeptide I′ was prepared from Boc-leucine.H_2O (27.5 g, 0.11 mole) and leucine methyl ester.HCl (20 g, 0.11 mole) by mixed anhydride coupling using N-methyl morpholine and isobutylchloroformate. After a standard work-up procedure the dip ptide I′ was obtained as white needles, 35.0 g (88%) from EtOAc/petrol bpt 40—60°, m.p. 132—133°

(2) Preparation of compound II'

The dipeptide I' (7.2g, 20 mmol) was dissolved in b nzen (120 ml, Na-dried). A solution of sodium dihydro-bis-(2-methoxyethoxy)aluminate (SDA, 70% in toluene, 41 ml) was added slowly with cooling. After addition, the solution was refluxed for ½ hr, cooled and poured into 0.5 M ice-cold citric acid solution. At pH 2.5 th aqueous solution was extract d with ether (4X) and the combin d extracts were discarded. The pH was adjusted to 9 with Na_2CO_3 solution and the aqueous solution was saturated with sodium chloride. Extraction with ether (4X), followed by drying (Na_2SO_4) of the combined organic phases, evaporation and crystallisation from petrol (40—60°) at −20° gave the reduced dipeptide II': 5.1 g (78%) as white needles. m.p. 59—60°

Nmr ($CDCl_3$) 9.05—9.15 (12H, d, 4 × CH_3) 8.75 (6H, m, 2 × $(CH_3)_2CH—CH_2$); 8.55 (9H, s, $(CH_3)_3CO$); 7.35 (5H, m, CH_2NH, CH_2OH); 6.05—6.85 (3H, m, 2 × α—CH and CH_2OH); 5.3 (1H, d, Boc NH—).

(3) Protection of compound II' with benzenesulphonyl.

The reduced compound II' (11.0 g, 34.7 mmole) in dioxan (100 ml) was added to a solution of $KHCO_3$ (21 g., 6 equiv.) in H_2O (100 ml). This mixture was cooled in ice and benzene-sulphonyl chloride (9.0 ml, 2 equiv.) added in dioxan (25 ml) with vigorous stirring. Stirred at 22° overnight. Poured into ether, washed with 2N NH_4OH (4X), H_2O (1X) 0.5M citric acid (2X to remove any unsulphonated material), H_2O (1X).

The protected compound III' was obtained as an oil. Nmr spectroscopy showed the presence of one benzenesulphonyl group. This material was used without further purification in the next stage:—

(4) Oxidation of compound III'

The material from the preceeding preparation was taken up in pyridine (50 ml), cooled in ice and $KMnO_4$ (11.0 g 70 mmole) in H_2O (50 ml) and pyridine (100 ml) added. Stirred for 42 hours at 20°. The MnO_2 precipitate was removed and the filtrate diluted with citric acid solution until acidic. Ether extraction at pH 5 removed product and starting material. The product IV' was obtained by (i) $NaHCO_3$ extraction—to remove strongly acidic by-products (ii) extracted with 30% v/v 0.880 ammonia solution (6X). The ammonia washes contained essentially pure IV'. Starting material remaining in the ether was re-oxidised for 42 hours and worked-up as above.

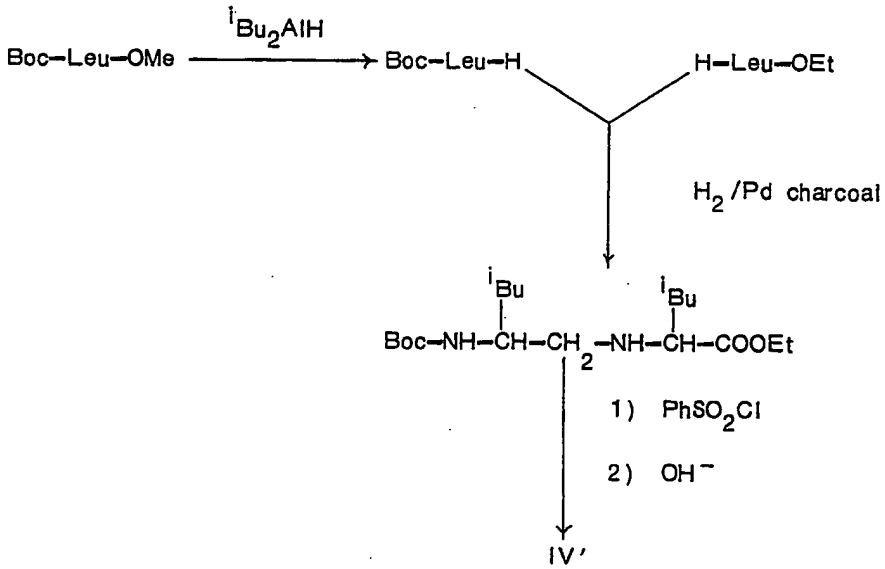
The total amount of IV' (isolated by acidifying the ammonia washes and extracting with $CHCl_3$) obtained was 2.34 g (20% based on II').

The material was a colourless foam Rf 0.41 by TLC on silica in benzene-dioxan-acetic acid (95:25:4).

NMR ($CDCl_3$): 8.9—9.3 (12H, m, 4 × CH_3); 8.2—8.8 (15H, m, $(CH_3)_3CO$ and 2 × $(CH_3)_2CH—CH_2$); 5.4—7.0 (4H, m, 2 × α—CH and $—CH_2—N—$); 2.0 and 2.4 (5H, m, $C_6H_5SO_2$), 1.2 (1H, br.s, CO_2H).

Alternatively, the reduced Leu-Leu analogue IV may be synthesized by the following method:

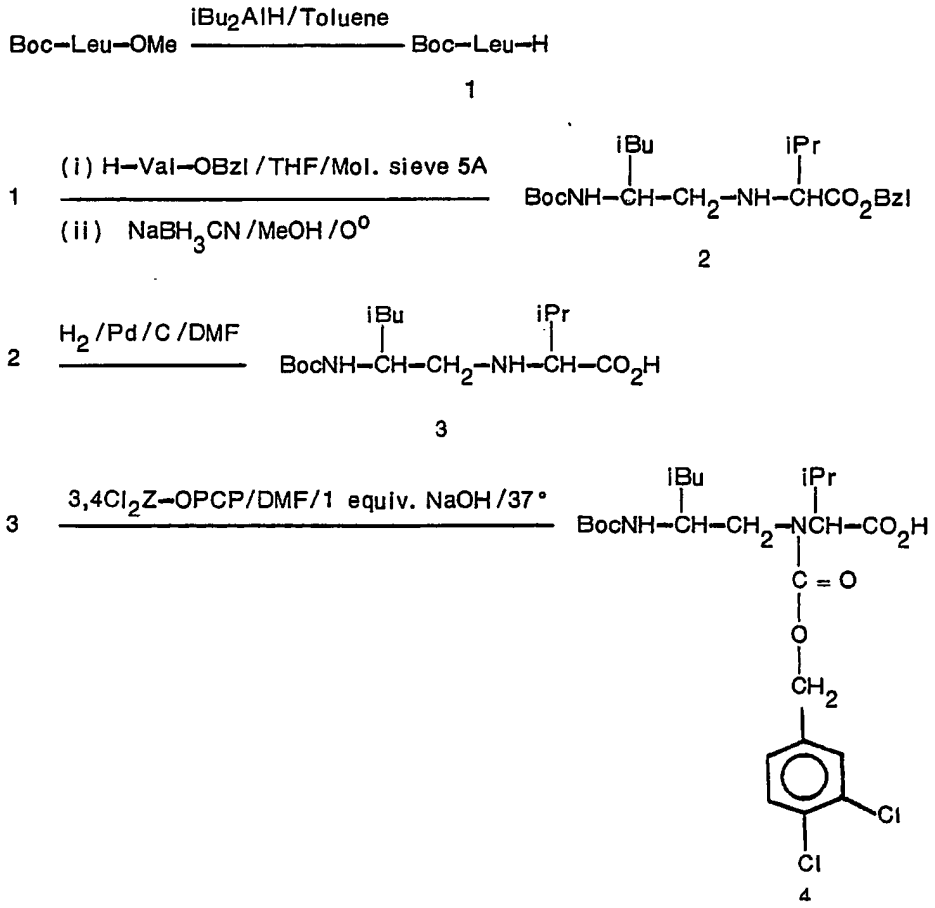
SCHEME 2



The following is a synth tic method for a reduced leu-Val isoster by a pr ferred rout .

SCHEME 3

Synthesis of the protected reduced isostere of L-leucyl-L-leucine



(1) Boc-L-Leucinal, 1

Boc-L-Leucine methyl ester (22.7 g, 90 mmoles) in dry toluene (250 ml) under N_2 was cooled to −78° and 25% di-isobutylaluminium hydride in toluene (130 ml, 225 mmoles) were added over 25 mins. keeping the temperature under −70°. The mixture was stirred for 15 mins. at −78° after completion of the addition, then MeOH (10 ml) was added cautiously. When effervescence ceased the mixture was poured into an ice-cold solution of Rochelle salt (100 ml of saturated solution + 600 ml H_2O). This mixture was shaken until an extractable solution was obtained. The toluene was separated and the aqueous phase re-extracted with ether (2 × 300 ml). Toluene and ether extracts were combined and dried (Na_2SO_4). The resulting oil was passed rapidly through a pad of silica gel in 15% EtOAc/petrol 40—60°. The crude aldehyde was obtained as an oil, weight 18.68 g. Nmr showed aldehyde content to be 85%, therefore yield of aldehyde: 15.9 g (83%).

Nmr ($CDCl_3$), τ: 0.45 (1H, s, CHO); 4.87 (H, br. d., Boc NH): 5.83 (1H, br. m., NH—CHCHO); 8.43—8.93 (12H, m, $(CH_3)_3C$, $(CH_3)_2CH.CH_2$); 9.0 and 9.1 (12H, 2 × d, $(CH_3)_2CH$)

TLC: (solvent 30% EtOAc/petrol 60—80°), Rf = 0.43.

(2) Boc-L-Leucyl-L-valine benzyl ester reduced isostere, 2

L-Valine-OBzl (10 mmoles, from EtOAc/1N $NaHCO_3$ partition of 3.8 g of p-toluene sulphonate salt) and Boc-L-Leucinal (2.54 g, 10 mmole aldehyd content) in dry t trahydrofuran (20 ml) stood over 5Å molecular si v (10 g) overnight. Sodium cyanoborohydride (630 mg, 10 mmoles) in MeOH (3 ml) was added with cooling, then left at room t mperatur for 30 mins. The mixtur was diluted with m thylene chloride (100 ml), filtered and evaporated to dryness. The r sidue was passed down a silica c lumn in 20% EtOAc/petrol (60—80°) to remove polar impurities. Isostere containing factions were combined. Crystallisation from petrol 60—80° at −20° gave large clusters of needles, 1.52 g (38%) m.p.

τ: 2.65 (5H, s, $OCH_2C_6H_5$); 6.35(1H, m, $NHCHCO_2Bzl$); 7.05 (1H, m, $NH—CHCH_2$); 7.45 (2H, m, $—CH_2NH—$); 8.25—8.90 (13H, m, $(CH_3)_3CO—$.) $(CH_3)_2$ $CHCH_2$ and $(CH_3)_2$ $CH—$); 9.05 and 9.15 (12H, 2 × s, 2 × $(CH_3)_2CH$).

TLC: (S lvent: 30% EtOAc/p trol 60/80°) Rf = 0.39.

(3) N-(2S)-t-Butyloxycarbonylamino-4-methylpentyl, N-(3,4-dichlorobenzyloxycarbonyl)-L-valine, 4

Boc-L-Leucyl-L-valine, benzyl ester-reduced-isostere (1.5 g 3.68 mmoles) in dimethylformamide (60 ml) was hydrogenated at STP over 5% Pd/C (150 mg). After 3½ hours the colloidal solution was flushed with nitrogen and 1M NaOH (3.8 ml, 1.05 equiv.) was added followed by 3,4-dichlorobenzyl pentachlorophenyl carbonate (1.92 g, 4.07 mmoles). The mixture was kept at 50° in a stoppered flask for 24 hours. and then evaporated to dryness. EtOAc was added and the Pd/C filtered off. The EtOAc solution was washed with 1M citric acid (2 x), H_2O (1 x), brine (1 x), and dried (Na_2SO_4).

The crude isostere *4* was chromatographed on silica-gel (Merck Keiselgel 60, 40—63 m) eluting with 2% MeOH/$CHCl_3$ to give the title compound as a colourless oil.

Nmr ($CDCl_3$), τ: 2.5—2.9 (3H, m, $C_6Cl_2H_3$); 3.3—3.8 (2H, br, BocNH and CO_2H); 4.85 and 4.95 (2H, 2 × s, $OCH_2—C_6Cl_2H_3$): 5.5—6.3 (2H, m, $NHCHCH_2$ and $—NCHCO_2H$); 6.5—7.2 (2H, br, 2 × d, $CHCH_2N—$); 8.2—8.9 (13H, m, $(CH_3)_3CO$, $(CH_3)_2CHCH_2$ and $(CH_3)_2CH—$); 8.9—9.4 (12H, m, 2 × $(CH_3)_2CH$)

TLC: (solvent 5% MeOH/$CHCl_3$) Rf = 0.32.

Examples

The following detailed Examples illustrate the invention.

The Examples are preceded by the preparation of Boc-Tyr [Bzl (2, 6 Cl_2)] —O-resin. (Reaction times marked * are convenient rather than necessary).

Preparation of Resin

Boc-Tyr [Bzl(2, 6 Cl_2)]—OH (1.65 g, 3.75 mmol) was dissolved in ethanol (20 ml) and water (5 ml) added. The pH was brought to 7.0 with cesium bicarbonate solution and the solvent evaporated *in vacuo*. The residue was treated twice with toluene and evaporated to remove the last traces of water leaving a white powder which was dried for several hours over phosphorus pentoxide. The residue was dissolved in DMF (65 ml), chloromethylated resin (10 g, 7.5 mequiv.) added and the reaction stirred at 37° for four days.

The resin was then filtered and washed thoroughly with DMF, DMF/water (9:1) and then DMF again. The resin was then resuspended in DMF (65 ml) and treated with acetic anhydride (2.36 ml, 25 mmol) and triethylamine (3.5 ml, 25 mmol) for 3 days.

The resin was filtered, washed thoroughly with DMF, DMF/water (9:1) and methanol and dried. The resin was then "defined" by shaking a suspension in dichloromethane and removing the particles slowest to float. The resin was then dried.

Yield 10.8 g.

Amino-acid analysis: (12N—HCl/propionic acid 1:1 130°, 2 hours) gave an incorporation of 0.11 mmol/g.

Example I

H-His-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH (H—76)

Boc-Tyr [Bzl(2,6 Cl_2)] —O-Resin (3g, 0.6 mmol) was washed with reagents in the following sequence: CH_2Cl_2 (3X) iPrOH (2X), CH_2Cl_2 (3X), 40% TFA/CH_2Cl_2 1 min then 20 min, CH_2Cl_2 (3X), iPrOH (2X), CH_2Cl_2 (3X), 40% TFA/CH_2Cl_2 1 min then 20 min, CH_2Cl_2 (3X) iPrOH (2X) CH_2Cl_2 (3X), 10% Et_3N/CH_2Cl_2 (2 × 2 min), CH_2Cl_2 (5 X). Boc-Val-OH (0.65 g, 3 mmol) was then coupled using DCCI (0.68 g, 3.3 mmol) and HOBt (0.92 g, 6 mmol) in DMF/CH_2Cl_2 (1:1) for 17 hours. The resin was then washed with DMF (3X), CH_2Cl_2 (3X), iPrOH (2X), CH_2Cl_2 (3X) 10% Et_3N/CH_2Cl_2 (2 min), CH_2Cl_2 (5X) then acetylated using acetylimidazole (0.66 g, 6 mmol) in DMF for 1 hour. The resin was then washed with DMF (3X) CH_2Cl_2 (3X) iPrOH (2X) and finally CH_2Cl_2 (3X).

The sequence of washes and reactions was repeated for the addition of each of the residues with the following modifications.

After deprotection of the Boc-Val-Tyr [Bzl (2,6 Cl_2)]—O-resin Boc-NH-CH (CH_2CHMe_2)-CH_2-$N(SO_2Ph)$-$CH(CH_2CHMe_2)$-CO_2H (0.42 g, 0.9 mmol) was coupled using DCCI (0.28 g, 1.35 mmol) and HOBt (0.275 g, 1.8 mmol) in DMF/CH_2Cl_2(1:1) for 18 hours, followed by acetylation using acetylimidazole (0.66 g, 6 mmol) in DMF for 1 hour.

After deprotection, Boc-His(Dnp)-OH (1.44 g, 3 mmol) was coupled using DCCI (0.68 g, 3.3 mmol) and HOBt (0.92 g, 6 mmol) for 2 hours, followed by acetylation for 1 hour.

Deprotection of the histidyl peptid was achieved using 50% TFA/CH_2Cl_2 instead of the usual 40% TFA/CH_2Cl_2. Boc-Ph -OH (0.796 g, 3 mmol) was coupled using DCCI (0.68 g, 3.3 mmol) and HOBt (0.92 g, 6 mmol) for 17½ hours*, followed by ac tylati n f r 1 hour.

Deprotecti n of the phenylalanyl peptide was achi ved using the usual 40% TFA/CH_2Cl_2. Boc-Pro-OH (0.646 g, 3 mmol) was coupled using DCCI (0.68 g, 3.3 mmol) and HOBt (0.92, 6 mmol) for 2 hours followed by acetylation for 1 hour.

After deprotection B c-His(Dnp)-OH (1.44 g, 3 mmol) was coupled using DCCI (0.68 g, 3.3 mmol) and

HOBt (0.92 g, 6 mmol) for 14½ hours* followed by acetylation for 1 hour.

The resin was then washed with DMF (3X), CH_2Cl_2(3X) iPrOH (2X), CH_2Cl_2(3X) and finally MeOH (3X) and dried to give 3.5353 g of product.

1.2 g of this material was treated with HF at 0° for 1½ hours in the presence of anisol (1.5 ml) then dried overnight over potassium hydroxide. The resin was then washed with DMF/water (1:1), acetic acid and finally acetic acid/water (1:1) to remove the peptide. These washes were combined and evaporated *in vacuo.*

The residue was dissolved in DMF (15 ml) and water (6 ml) thioethanol (5 ml) added and the pH of the solution brought to 8.0 with sodium carbonate. The reaction was stirred overnight the solvent evaporated and the residue applied to a Sephadex G 25 column (72 × 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 27—46 were combined and the solvent evaporated *in vacuo* and dried. Then 90% of the residue was taken (for the rest see Example III) and dissolved in anhydrous ammonia (100 ml) and small portions of sodium wire added until a permanent blue colour was achieved for 15 seconds. The ammonia was allowed to evaporate and the residue dried.

The residue was applied to a Sephadex SPC25 column (77 × 1.6 cms) eluted with 30% acetic acid at 40 mls/hr, with a sodium chloride gradient from 0.01M to 1M over two days collecting 6.6 ml fractions.

The product was contained in fractions 100—104. These were pooled, evaporated and the residue dissolved in glacial acetic acid and filtered to remove the sodium chloride. The solution was evaporated and desalted on a Sephadex G25 column (72 × 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 32—6 were pooled, evaporated, transferred to a vial and lyphilised.

Yield 13.4 mg

Product $C_{52}H_{74}O_9N_{12}$ MW. 1011,25
T.l.c. (silica) Rf 0.15 EtOAc/Pyr/AcOH/H_2O 40:20:6:11
Rf 0.40 nBuOH/Pyr/AcOH/H_2O 30:20:6:24
T.l.e. pH 2.1 1000V 30 min mobility 8.3 cm.
pH 6.5 1000V 30 min mobility 7.5 cm.
AAA 6N HCl + phenol 110°, 40 hours, peptide content 72%
His: 1.97; Pro: 1.01; Val: 1.02; Tyr: 0.98; Phe: 1.01.

Example II

H—Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH (H—79)

Fractions 80—84 of the SPC 25 Sephadex Column from the previous synthesis were combined, evaporated and the residue dissolved in glacial acetic acid and filtered to remove sodium chloride. The solution was evaporated and the product desalted on a Sephadex G25 column (72 × 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 32—9 were pooled, evaporated, transferred to a vial and lyophilised.

Yield 23.6 mg

Product $C_{46}H_{67}O_8N_9$ MW 874,10
T.l.c. (silica) Rf 0.29 EtOAc/Pyr/AcOH/H_2O 40:20:6:11
Rf 0.46 nBuOH/Pyr/AcOH/H_2O 30:20:6:24
T.l.e. pH 2.1 1000V 30 min mobility 7.5 cms
pH 6.5 1000V 30 min mobility 8.3 cms
AAA 6N HCl + phenol, 110°, 40 hours, peptide content 85%
His: 0.97; Pro: 1.08; Val: 0.99; Tyr: 0.97; Phe: 1.00.

The above example illustrates how Y in formulae (V), (VA) and (VB) may be absent.

Example III

H-His-Pro-Phe-His-Leu-reduced (SO_2Ph)-Leu-Val-Tyr-OH (H—78)

In the synthesis of compound H76 10% of the residue from the Sephadex G25 column after the HF and thioethanol treatments of the resin was kept.

This material was applied to a Sephadex SPC25 column (77 × 1.6 cm) eluted with 30% acetic acid at 20 mls/hr with a sodium chloride gradient from 0.01M to 1M over 2 days collecting 6.6 ml fractions.

The product was contained in fractions 74—7. These were pooled, evaporated, dissolved in glacial acetic acid and filtered to remove sodium chloride. The solution was then evaporated and desalted on a Sephadex G25 column (72 × 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 31—4 were pooled evaporated, the residue transferred to a vial and lyophilised.

Yield 0.6 mg

Product $C_{58}H_{78}O_{11}N_{12}S$ MW: 1151.40
T.l.c. (silica) Rf 0.31 EtOAc/Pyr/AcOH/H_2O 40:20:6:11
T.l.e. pH 2.1 1000v 30 min mobility 5.4 cms.
AAA 6N HCl + phenol, 40 hrs, 110°, peptide content 64%
His: 1.93; Pro: 1.08; Val: 1.05; Tyr: 0.96; Phe: 0.97.

Example IV

H-DHis-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH, (H—77)

The Boc-Tyr [Bzl(2,6,Cl_2)] —O-Resin (3g, 0.6 mmol) was deprotected and Boc-Val-OH (0.65 g, 3.0 mmol) was coupled using DCCI (0.68 g, 3.3 mmol) and HOBt (0.92 g, 6 mmol) for 22 hours* then acetylated with acetylimidazole (0.66 g, 6 mmol) for 1 hour.

After deprotection, Boc-Leu-reduced (SO_2Ph)-Leu-OH, IV, (0.42 g 0.9 mmol) was coupled using DCCI (0.28 g, 1.35 mmol) and HOBt (0.275 g, 1.8 mmol) for 20 hours, then acetylated for 1 hour.

After deprotection Boc-His(Dnp)-OH (1.44 g, 3 mmol) was coupled using DCCI (0.68 g, 3.3 mmol) and HOBt (0.92 g, 6 mmol) for 22 hours*, then acetylated for 1 hour.

After deprotection, this time with 50% TFA/CH_2Cl_2 Boc-Phe-OH (0.796 g, 3 mmol) was coupled with DCCI (0.68 g, 3.3 mmol) and HOBt (0.92 g, 6 mmol) for 19 hours* then acetylated for 1 hour.

After deprotection Boc-Pro-OH (0.646 g, 3 mmol) was coupled using DCCI (0.68 g, 3.3 mmol) and HOBt (0.92 g, 6 mmol) for 3 hours then acetylated for 1 hour.

The peptide was again deprotected and coupled with Boc-D-His(Boc)-OH (0.91 g, 2.56 mmol using DCCI (0.58 g, 2.82 mmol) and HOBt (0.78 g, 5.1 mmol) for 2 hours, then acetylated for 1 hour.

The resin was washed with DMF (3X) CH_2Cl_2 (3X) iProH (2X) CH_2Cl_2 (3X) and finally MeOH (3X) and dried to give 3.6563 g of product.

1.2 g of this material was treated with HF at 0° for 1¼ hours in the presence of anisole (1.5 ml) then dried overnight over potassium hydroxide. The resin was then washed with DMF, DMF/H_2O (1:1) acetic acid and finally acetic acid/water (1:1) to remove the peptide. These washes were combined and evaporated *in vacuo*.

The residue was dissolved in DMF (15 ml) and water (6 ml), thioethanol (5 ml) added and the pH of the solution brought to 8.0 with sodium carbonate solution. The reaction was stirred overnight the solvent evaporated and the residue applied to a Sephadex G 25 column (72 × 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 27—46 were combined and the solvent evaporated *in vacuo* and dried.

90% of the residue was dissolved in anhydrous ammonia (100 ml) and small portions of sodium wire added until a permanent blue colour was achieved for 15 seconds. The ammonia was allowed to evaporate and the residue dried.

The residue was applied to a Sephadex SPC 25 column (77 × 1.6 cms) eluted with 30% acetic acid at 20 mls/hr with a sodium chloride gradient 0.01 M to 1M over 2 days collecting 6.6 ml fractions.

The product was contained in fractions 88—92. These were pooled, evaporated and the residue dissolved in glacial acetic acid and filtered to remove the sodium chloride. The solution was evaporated and desalted on a Sephadex G25 column (72 × 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 32—41 were pooled, evaporated, transferred to a vial and lyophilised.

Yield 46.8 mg

Difference in yields between "L-His" and "D-His" compounds was accounted for by a lower incorporation of the isostere in the "L" case and less removal of the histidine with Na/NH_3.

Product $C_{52}H_{74}O_9N_{12}$ MW 1011,25
Tlc (silica Rf 0.18 EtOAc/Pyr/AcOH/H_2O 40:20:6:11
Tle pH 2.1 1000V 30 min mobility 77 cm
pH 6.5 1000V 30 min mobility 7.9 cm
AAA 6N-HCl + phenol, 110°, 40 hours, peptide content 93%
His: 1.98, Pro: 1.00; Val: 1.08; Tyr: 0.97; Phe 0.97

Example V

H-His-Pro-Phe-His-Leu-reduced-Val-Ile-His-OH (H 113)

The method is generally that of Example I above but illustrates formula (VB).

Preparation of Boc-His (DNP)-O-Resin (AH/30/83)

Boc-His(DNP)- OH. (4.74g, 11.25 mmol) was dissolved in ethanol (60ml) and a solution of cesium bicarbonate (2.18g, 11.25mmol) in water (15 ml) added. The solvent was evaporated *in vacuo* and the residue treated four times with toluene and evaporated to remove water before finally drying overnight over phosphorus pentoxide. The residue was dissolved in DMF (175 ml), chloromethylated resin (30g, 22.5 m-equiv.) added and the reaction stirred at 37° for five days.

The resin was filtered off and washed thoroughly with DMF, DMF/water (9:1) and then DMF again. It was resuspended in DMF (175 ml) and treated with acetic anhydride (7.08 ml, 75 mmol) and triethylamine (10.5ml, 75 mmol) overnight.

Th resin ster was filt r d, washed thor ughly with DMF, DMF/water (9:1) and methanol and dried. It was then "de-fined" by shaking it in dichloromethane and removing the particles in the supernatant. Finally, the resin was dried. Yield: 30.45g. A trial coupling with Boc-Ala-OH, followed by amino-acid analysis (after hydrolysis with 12N-HCl/propionic acid 1:1, 130°, 2hrs) gave an incorporation of 0.2mmol/.

Coupling to Resin Ester

Boc-His(DNP)-O-Resin (2.5g 0.55 mmol) was deprotected with 50% TFA/CH_2Cl_2 and Boc-Ile-OH (0.748g 3mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6.0mmol) for 2 hours, th n acetylated with acetyl imidazole (0.55g, 5mmol) overnight.

After deprot ction with 40% TFA/CH_2Cl_2, Boc-Leu-reduced (3,4-Cl_2-Z)-Val-OH, *4* (0.343g, 0.66 mmol) was coupled using DCCI (0/15g, 0.73mmol) and HOBt (0.20g, 1.32 mmol) for 16 hours, th n actylated f r 1 hour.

After deprotection, Boc-His(DNP)-OH (1.26g, 3.0 mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6mmol) for 2 hours, then acetylated for 1 hour.

After deprotection, again with 50% TFA/CH_2Cl_2, Boc-Phe-OH (0.796g, 3mmol) was coupled with DCCI (0.68g, 3.3mmol) and HOBt (0.92g, 6mmol) for 3 hours then acetylated overnight.

After deprotection, Boc-Pro-OH (0.646g, 3mmol) was coupled using DCCI (0.68g, 3.3mmol) and HOBt (0.92g, 6mmol) for 2 hours then acetylated for 1 hour.

The peptide was again deprotected and coupled with Boc-His (DNP)-OH (1.26g, 3mmol) using DCCI (0.68g, 3.3mmol) and HOBt (0.92g, 6mmol) for 2 hours, then acetylated overnight.

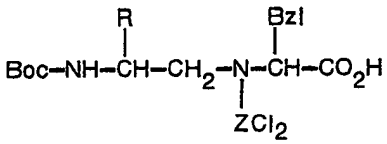
The peptide resin ester was washed with DMF (3X), CH_2Cl_2 (3X) iProH (2X), CH_2Cl_2 (3X) and finally MeOH (3X) and dried. It was then treated with HF at 0° for 1¼ hours in the presence of anisole (4ml) and dried overnight over potassium hydroxide. The resin was washed with DMF, acetic acid and acetic acid/water (1:1) to remove the peptide. The washes were combined and evaporated *in vacuo*.

The residue was dissolved in DMF (60 ml) and water (24ml), thioethanol (10ml) was added and the pH of the solution brought to 8.0 with sodium carbonate solution. The reaction mixture was stirred overnight, the solvent evaporated and the residue applied to a Sephadex G25 column (77 × 2.5cms). It was eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 34—53 were combined and the solvent evaporated *in vacuo* and dried.

Product $C_{49}H_{72}O_3N_{14}$ MW 985,21
Tlc (silica) Rf = 0.63 in EtOAc-Py-AcOH-H_2O (15:20:6:11)
AA analysis in accordance with calculated composition.

Examples VI — IX

These Examples illustrate formula (VA). The methods disclosed above are applied to condensing Boc-Phe-H or Boc-Leu-H with H-Phe-OBzl, reducing the imine link, deprotecting at the carboxyl terminus and protecting the nitrogen of the reduced peptide link to give:



12 R = Bzl
12a R = iBu ZCl_2 = 3,4-dichloro benzyloxy carbonyl

This Phe-reduced-Phe or Leu-reduced-Phe analogue is then used as follows:

— *VI* Use of *12* (Phe-reduced-Phe) in an analogue otherwise as H—77 (see Example IV)
— *VII* Use of *12a* (Leu-reduced-Phe) in an analogue otherwise as H—77 (see Example IV)
— *VIII* Use of *12* in an analogue as H—76 (Example I), viz:

H-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-OH (H 110)

6 7 8 9 10 11 12 13

— *IX* Use of *12a* in an analogue as H—76 (Example I), viz:

H-His-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH (H 115)

6 7 8 9 10 11 12 13

Example X

This Example illustrates formula (VB), the method of Exampl V being used but with the Tyr resin of Examples I to IV, to give:

H-His-Pro-Phe-His-Leu-reduced-Val-Ile-Tyr-OH (H 116)

6 7 8 9 10 11 12 13

Example XI

Use of methods disclosed herein to give:

H-Pro-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-Lys-OH (H 108)

5 6 7 8 9 10 11 12 13 14

this being an analogue illustrating the non-criticality of the terminal portions of the chain allowing X and W in formulae (V), (VA) and (VB) to represent further residues. It is a further example of formula (VA).

Example XII

Use of the methods disclosed herein to give:

H-His-Pro-Phe-His-Leu-reduced-Val-Val-Tyr-OH (H 117)

6 7 8 9 10 11 12 13

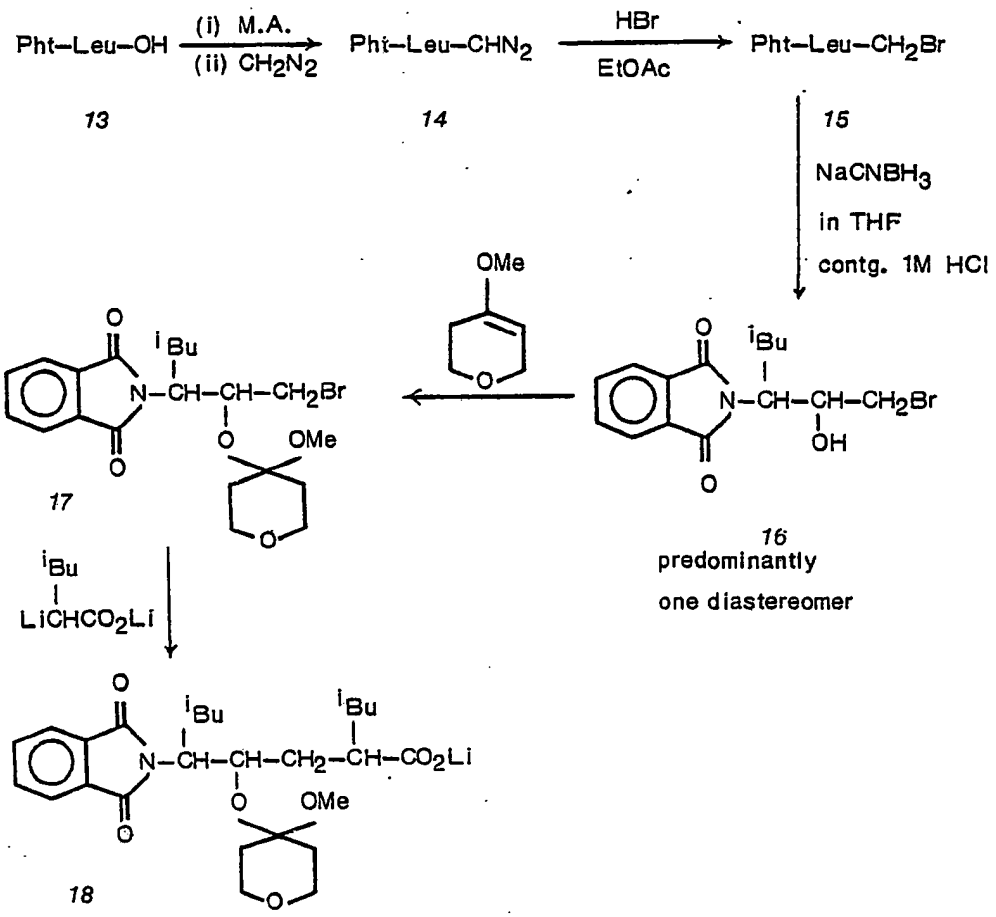
a compound of value in its relation to the Leu-Val structure at 11, 12 in human renin substrate.

The following Example illustrates the synthesis of hydroxy and keto isosteres.

Example XIII

The reaction scheme below, also suitable for other hydroxy dipeptide analogues, was used to synthesise an N-terminal and hydroxy-group protected Leu-Leu hydroxy isostere *18*.

Scheme 4



M.A. = isobutyl chloroform/triethylamine Pht = phthaloyl

The resulting N-terminal phthaloyl protected, —OH protected hydroxy isostere of Leu-Leu can be coupled direct for example to valyl tyrosine, followed by removal of the phthaloyl group, coupling direct to a suitable tri or tetrapeptide, and deprotection at the —OH group by mild acid hydrolysis, to give for example analogues corresponding to H—76 (Example 1), H—79 (Example 2), H—77 (Example 4). Alternatively the phthaloyl group may be removed by treatment with hydrazine and a new protective group, e.g. benzyloxycarbonyl or t-butyloxycarbonyl attached prior to coupling. The methods used after the preparation of the protected hydroxy isosteres are those of the peptide synthesis art, well known in themselves and exemplified in detail herein. The compounds specifically prepared are:

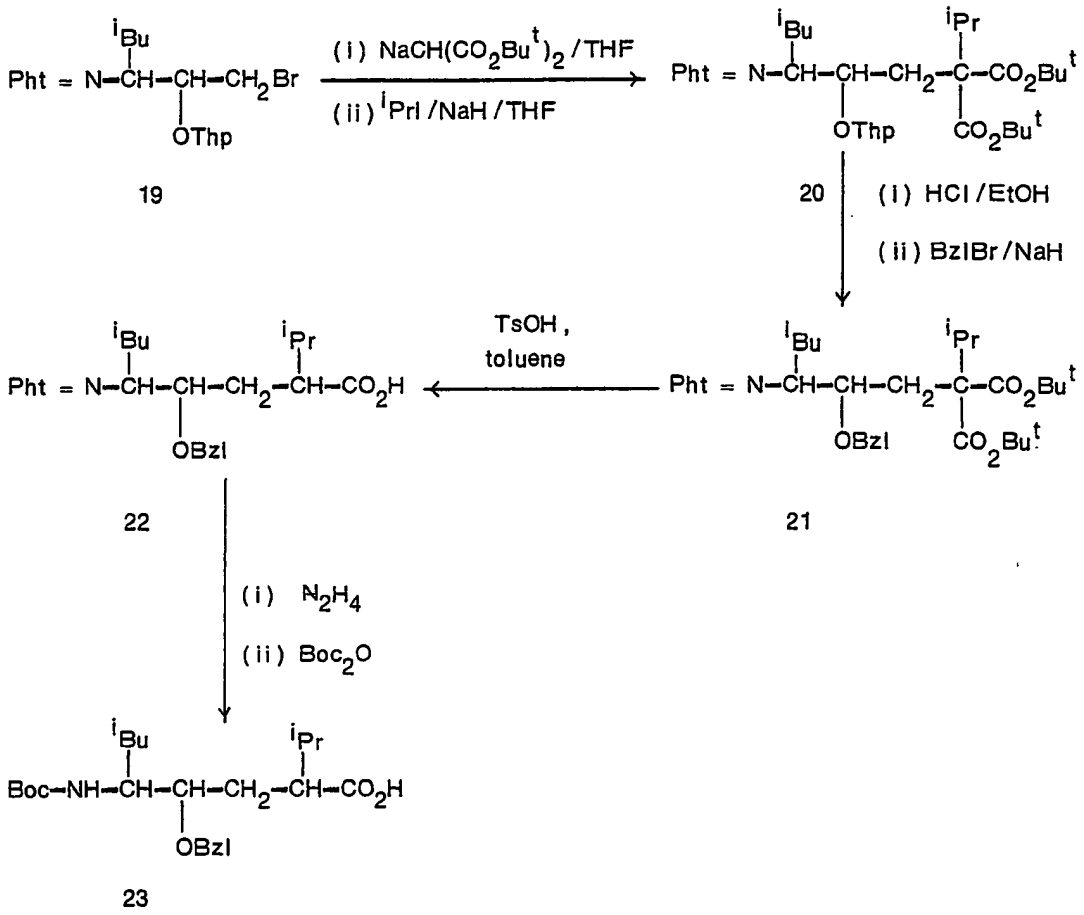
a) H-His-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH
b) H-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH
c) H-DHis-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH

Example XIV

The alternative and preferred reaction scheme below, also suitable for other dipeptide hydroxy isosteres was used to synthesise an N-terminal and hydroxy-group protected Leu-Val hydroxy isostere *23.*

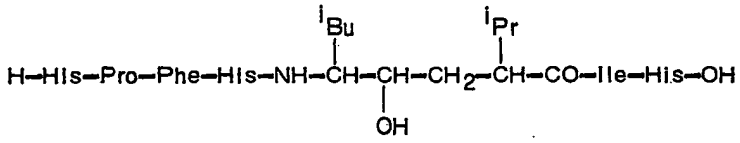
SCHEME 5

Pht = Phthaloyl Thp = Tetrahydropyranyl



In the above scheme the protected bromohydrin *19* is obtained in the sam way as the corresponding interm diate *17* in Scheme 4, and is subjected to malonic ester synthesis and alkylation with isopropyl iodide to give the malonic est r derivative *20.* Protection on the hydroxyl function is changed from Thp to Bzl to yield *21* and the latter is subjected to protonolysis and decarboxylation. In the resulting isostere acid

22 amino protection is changed from Pht to Boc yielding the prot cted isostere *23* which is suitable for incorporation into an octapeptide analogue by the usual methods of solid phase peptide synthesis to give for example the octapeptid anal gue:

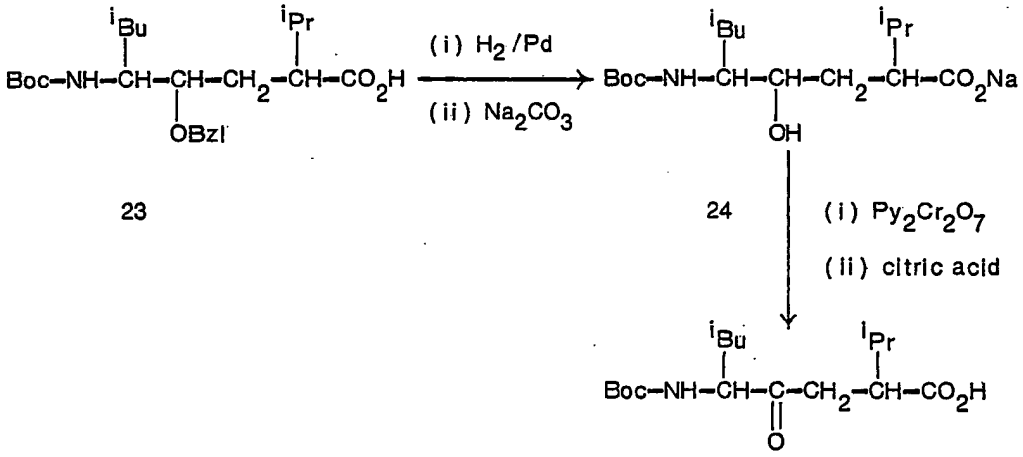


This is a Leu-hydroxy-Val isostere.

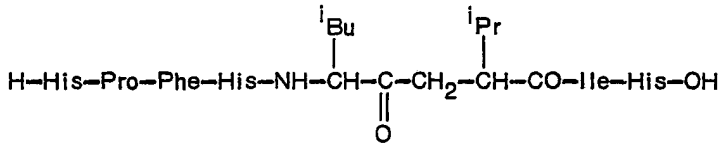
Example XV

Keto isosteres may be prepared for example by the method of published U.K. Specification No. 1 587 809 (U.S. 4 242 256) of R. Sharpe and one of the present inventors M. Szalke, to which reference may be made. Alternatively they may be prepared as disclosed herein and in particular, in the present Example, from the final product *23* of scheme 5 as in the scheme below:—

SCHEME 6

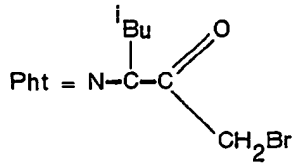


In the above scheme the benzyl protection is selectively removed from the hydroxyl function of *23* and the free acid is converted into its sodium salt *24*. The latter is subjected to oxidation by pyridinium dichromate and acidification to give the partially protected keto isostere acid which is ready for incorporation into an octapeptide analogue by the usual methods of solid phase synthesis, for example the analogue:

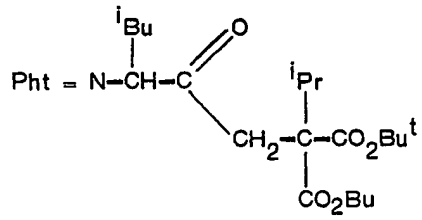


This is a Leu-keto-Val isoster .

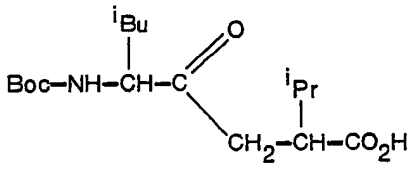
Alternativ ly th keto isost re may be prepared directly by a modified version of scheme 5 wherein the bromohydrin *19* is replaced by the bromoketone *15* (scheme 4), which is:



giving the compound



which is successively treated with TsOH, toluene and i) N_2H_4, ii) Boc_2O to give:



ACTIVITY IN VITRO

Preliminary activity test results in the human renin-renin substrate reaction in vitro are given in the table below, with comparative figures for the tetrapeptide analogue (III). The test is based on the methods described by J. A. Millar et al. in Clinica Chimica Acta (1980) *101* 5—15 and K. Poulsen and J. Jorgensen in J. Clin. Endocrinol. Metab. (1974) *39* 816.

It is based on the measurement, by radioimmunoassay, of Angiotensin-I released from human renin substrate by human renin in human plasma. The inhibitor is dissolved in 0.01 N HCl (10 µl) containing EDTA, and angiotensin-I antibody (15 µl) in 3M-Tris/HCl buffer (pH 6.9).

After incubation at 37°C for 0—120 mins., the enzymic reaction is quenched by the addition of ice-cold 0.25M Tris/HCl buffer (pH 7.4) containing 0.01% of bovine serum albumin. 125I-labelled angiotensin-I is added, followed by equilibration at 4°C for 48 hours. Free and bound ligand are separated by the addition of dextran-coated charcoal, and the amount of bound radio-ligand determination in a gamma counter.

The results for the renin inhibitory activities of the present compounds thus tested, expressed as the IC_{50} (the molar concentration required to cause 50% inhibition), are as follows:

Analogue	IC_{50}
Example I (H—76)	1.0 µM
Example II (H—79)	17.0 µM
Example III (H—78)	1.5 µM
Example IV (H—77)	1.0 µM
Example V (H—113)	0.26 µM **
Example VI	*
Example VII	*
Example VIII (H—110)	1.3 µM
Example IX (H—115)	2.5 µM
Example X (H—116)	0.20 µM
Example XI (H—108)	0.05 µM
Example XII (H—117)	*
Example XIII a)	*
b)	*
c)	*
Example XIV	*
Example XV	*

* Preliminary indication of comparable activities.
** The corresponding non-isosteric peptide has for example been tested and shows a potency over three orders of magnitude less (IC_{50} 400 µM)
(Comparative) III 822 µM

These are most notable results, showing a potency, in the reduction of renin activity remaining in the plasma in the presence of the analogue, several orders of magnitude greater than the previously proposed tetrapeptide analogue.

ACTIVITY IN VIVO

The activity tests below are in animals but indicate corresponding activity in man.

In *in vitro* studies, in plasma for both normal and sodium-depleted dogs, the compound H—77 (Example IV) inhibited renin. In *in vivo* studies H—77 was infused into normal conscious sodium-depleted dogs at rates of 0.01, 0.1, 1 and 10 mg/kg/hr. A maximum fall in blood pressure, plasma renin (PR) angiotensin-I (A—I) and antiogensin-II (A—II) levels was obtained within 10 minutes at doses of 1 and 10 mg/kg/hr. When the infusion was stopped, blood pressure returned to baseline levels 30 minutes after the 1 mg/kg/hr. dose, but more slowly after the 10 mg/kg/hr. dose.

In the claims below it will be understood that compounds may be in the form shown or in protected or salt form at NH_2, COOH, OH or other active groups and in particular as their physiologically acceptable acid addition salts at basic centres. Further as already noted herein general reference to amino acids and amino acyl residues and side chains is to be taken as reference to such whether naturally occurring in proteins or not and to both D- and L- forms, and amino is to be taken as including imino except where an aromatic acid, residue or side chain is specified.

Claims for the Contracting States: BE CH DE FR GB IT LI LU NL SE

1. A polypeptide analogue of the formula:

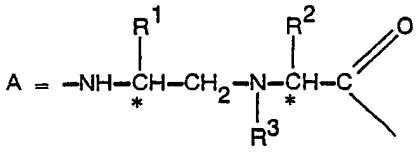
X-Y-Pro-Phe-His—A—B—Z—W (V)
6 7 8 9 10,11 12 13

where:—

Pro, Phe and His may be in substituted form;

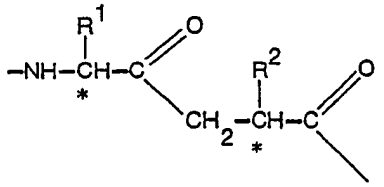
X = H; or an acyl or other N-protecting group e.g. acetyl, pivaloyl, *t*-butyloxycarbonyl (Boc), benzoyl or lower alkyl (primarily C_1—C_5); or an L- or D-amino- acyl residue, which may itself be N-protected similarly;

Y = D- or L-His or other D- or L- basic or aromatic amino-acyl residue, or is absent;



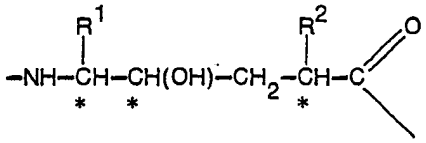
(VI) "reduced" isostere bond

or



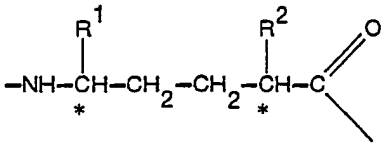
(VII) "keto" isostere bond

or



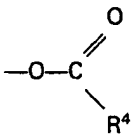
(VIII) "hydroxy" isostere bond

or



(IX) "hydrocarbon" isostere bond

where the configuration at asymmetric centres * is either R or S, where in VIII the hydroxy group may be present as such or protected in ether —OR^4 or ester



form where R^4 is as given under W below and where

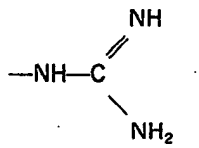
R_1 and R_2, the same or different = ^{i}Pro (isopropyl), ^{i}Bu (isobutyl), Bzl (benzyl) or other lipophilic or aromatic amino-acid side chain

R^3 = −H; lower alkyl (C_1—C_5); or —SO_2Ph, —$SO_2C_6H_4CH_3$(p), Boc, formyl or other N-protecting group;

B = D- or L- Val or Ile or other D- or L- lipophilic amino-acyl residue;

Z = D- or L- Tyr, Phe, His or other L- or D-aromatic amino-acyl residue; and

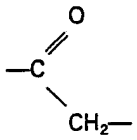
W = —OH as such or in protected ester form as —OR^4 where R^4 = lower alkyl primarily C_1—C_5 and particularly ^{t}Bu, or cycloalkyl primarily C_3—C_7, or Bzl, or other ester forming group; or —NH_2 as such or in protected amide form as —NHR^5 or —$N(R^5)_2$ (where R^5 = an N-protecting or other substituent group .g. lower alkyl as for R^4 and $(R^5)_2$ = two such or e.g. cycloalkyl, primarily C3—C_7) or as —NH—$(CH_2)_n$—Q (where n = 2 to 6 and Q = NH_2 or



and wherein any of the hydrogens attached to nitrogen may be substituted by R^5 or $(R^5)_2$); an L- or D- serine or lysine, arginine or other basic aminoacyl residue as such or in amide form, substituted amide form or ester form e.g. containing a group or groups as given for R^4 and R^5 above as the case may be; or an amino alcohol residue derived therefrom as such or protected in ester or ether form e.g. containing a group as given for R^4 above or

Z + W = an alcohol derived from L- or D-Tyr, Phe, His or other L- or D- aromatic amino-acyl residue as such or protected in ester or ether form as above;

such polypeptide being in the above form or modified by isosteric replacement of one or more remaining peptide bonds by reduced, $—CH_2—NH—$, keto,



hydroxy, $—CH(OH)—CH_2—$, or hydrocarbon, $—CH_2—CH_2—$ isosteric links and further being in free form or in protected form at one or more remaining peptide, carboxyl, amino, hydroxy or other reactive groups.

2. A polypeptide analogue according to claim 1, of the formula:

X-Y-Pro-Phe-His—A—Val—Z—W (VA)

6 7 8 9 10,11 12 13

where

X, Y, Pro, Phe and His are as in claim 1

A is as in claim 1 except that R^1 and R^2, the same or different = ^{t}Bu (isobutyl) or Bzl (benzyl) or other lipophilic or aromatic amino-acid side chain R^3 = —H; or $—SO_2Ph$, $—SO_2C_6H_4CH_3(p)$, Boc, formyl or other N-protecting group

Z = Tyr, Ph or other L- or D-aromatic amino-acyl residue;

W = —OH as such or in protected ester form as $—OR^4$ where R^4 = lower alkyl (primarily C_1—C_5 and particularly ^{t}Bu), or Bzl, or other ester forming group; or $—NH_2$ as such or in protected amide form as $—NHR^5$ or $—N(R^5)_2$ (R^5 = an N-protecting group e.g. lower alkyl as for R^4; $(R^5)_2$ = two such or e.g. cyclo-alkyl, primarily C_3—C_7) or an L- or D- amino-acyl residue e.g. a serine or basic amino-acyl residue as such or in amide form or in protected amide or ester form e.g. containing a group or groups as given for R^4 or R^5 above as the case may be; or an amino acid alcohol residue derived therefrom as such or protected in este or ether form e.g. containing a group as given for R^4 above or

Z + W = an alcohol derived from Tyr or Phe or other L- or D- aromatic amino acyl residue as such or protected in ester or ether form as above.

3. A polypeptide analogue, according to claim 1, of the formula:

X-Y-Pro-Phe-His—A—Ile—Z—W (VB)

6 7 8 9 10,11 12 13

where

X, Y, Pro, Phe and His are as in claim 1

A as in claim 1 except that R_1 = ^{t}Bu (isobutyl) or Bzl (benzyl) or other lipophilic or aromatic amino-acid side chain, R^2 = ^{i}Pr (isopropyl) and R^3 = —H; or $—SO_2Ph$, $—SO_2C_6H_4CH_3(p)$, Boc, formyl or other protecting group

Z is as in claim 1

W is as in claim 2 or

Z + W = an alcohol derived from the aromatic residues specified for Z in claim 1, as such or protected in ester or eth r form as specified ther in.

4. A polyp ptid analogue as any one of claims 1 to 3, modified by isosteric r placement, as s t out therein, at one or both of the Pro-Phe or Phe-His links.

5. A polypeptide analogue as any one of claims 1 to 4 wherein th isosteric replacement at at least the 10,11 position is of th "reduced" kind.

6. A polypeptide analogue as any one of claims 1 to 4 wherein the isosteric replacement at at least the 10,11 position is of the "hydroxy" kind.

7. The compound: H-His-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH.

8. The compound: H-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH.

9. The compound: H-His-Pro-Phe-His-Leu-reduced (SO_2Ph)-Leu-Val-Tyr-OH.

10. The compound: H-DHis-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH.

11. The compound: H-His-Pro-Phe-His-Leu-reduced-Val-Ile-His-OH.

12. The compound: H-DHis-Pro-Phe-His-Phe-reduced-Val-Tyr-OH.

13. The compound: H-DHis-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH.

14. The compound: H-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-OH.

15. The compound: H-His-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH.

16. The compound: H-His-Pro-Phe-His-Leu-reduced-Val-Ile-Tyr-OH.

17. The compound: H-Pro-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-Lys-OH.

18. The compound: H-His-Pro-Phe-His-Leu-reduced-Val-Val-Tyr-OH.

19. The compound: H-His-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH.

20. The compound: H-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH.

21. The compound: H-DHis-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH.

22. The compound: H-His-Pro-Phe-His-Leu-hydroxy-Val-Ile-His-OH.

23. The compound: H-His-Pro-Phe-His-Leu-keto-Val-Ile-His-OH.

24. A polypeptide analogue according to any one of claims 1 to 23, for use in a diagnostic test for high-renin states, blood pressure falling most when renin is high, or in a surgical prognostic test for reno-vascular hypertension (renal artery stenosis), by administration of said polypeptide analogue followed by monitoring of blood pressure.

25. A polypeptide analogue according to any one of claims 1 to 23, for use in the long and short term treatment of heart failure and all forms of hypertension particularly those associated with high serum renin levels, by administration of a renin-inhibiting amount of said polypeptide analogue.

26. A polypeptide analogue according to any one of claims 1 to 23, for use as a medicament, the dosage thereof being 0.001 to 10 mg/kg body weight daily, preferably 0.01 to 1.0 mg, of said polypeptide analogue.

27. A polypeptide analogue according to any one of claims 1 to 23, when in the form of a composition with a pharmaceutically acceptable diluent or carrier.

28. The composition of claim 27, in unit dosage form containing the amounts of analogue set out in claim 26 or sub-multiple thereof.

Claims for the Contracting State: AT

1. A process for preparing polypeptide analogues of the formula:

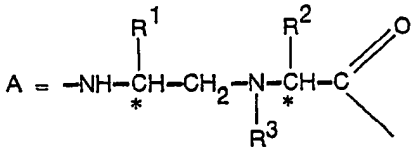
$$\underset{6\ 7\ \ 8\ \ 9\ 10,11\ 12\ 13}{X\text{-}Y\text{-}Pro\text{-}Phe\text{-}His\text{—}A\text{—}B\text{—}Z\text{—}W} \qquad (V)$$

where:—

Pro, Phe and His may be in substituted form;

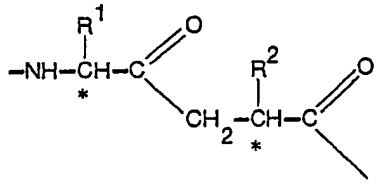
X = H; or an acyl or other N-protecting group e.g. acetyl, pivaloyl, *t*-butyloxycarbonyl (Boc), benzoyl or lower alkyl (primarily C_1—C_5); or an L- or D- amino- acyl residue, which may itself be N-protected similarly;

Y = D- or L-His or other D- or L- basic or aromatic amino-acyl residue, or is absent;



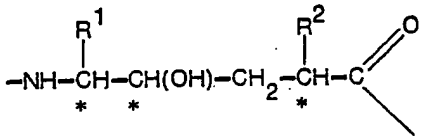
(VI) "reduced" isostere bond

or



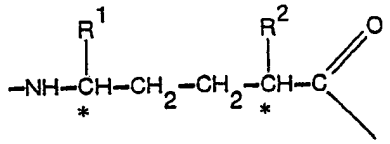
(VII) "keto" isostere bond

or



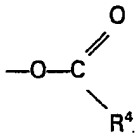
(VIII) "hydroxy" isostere bond

or



(IX) "hydrocarbon" isostere bond

where the configuration at asymmetric centres * is either R or S, where in VIII the hydroxy group may be present as such or protected in ether —OR^4 or ester



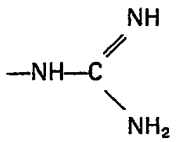
form where R^4 is as given under W below and where R^1 and R^2, the same or different = ^{i}Pro (isopropyl), ^{i}Bu (isobutyl), Bzl (benzytl) or other lipophilic or aromatic amino-acid side chain

R^3 = —H; lower alkyl (C_1—C_5); or —SO_2Ph, —$SO_2C_6H_4CH_3$(p), Boc, formyl or other N-protecting group;

B = D- or L- Val or Ile or other D- or L- lipophilic amino-acetyl residue;

Z = D- or L- Tyre, Phe, His or other L- or D-aromatic amino acyl residue; and

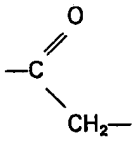
W = —OH as such or in protected ester form as —OR^4 where R^4 = lower alkyl primarily C_1—C_5 and particularly ^{t}Bu, or cycloalkyl primarily C_3—C_7, or Bzl, or other ester forming group; or —NH_2 as such or in protected amide form as —NHR^5 or —$N(R^5)_2$ (where R^5 = an N-protecting or other substituent group e.g. lower alkyl as for R^4 and $(R^5)_2$ = two such or e.g. cycloalkyl, primarily C_3—C_7) or as —NH—$(CH_2)_n$—Q or —$NR^5(CH_2)_n$—Q (where n = 2 to 6 and Q = NH_2 or



and wherein any of the hydrogens attached to nitrogen may be substituted by R^5 or $(R_5)_2$); an L- or D- serine or lysine, arginine or other basic aminoacyl residue as such or in amide form, substituted amide form or ester form e.g. containing a group or groups as given for R^4 and R^5 above as the case may be; or an amino alcohol residue derived therefrom as such or protected in ester or ether form e.g. containing a group as given for R^4 above or

Z + W = an alcohol derived from L- or D- Tyr, Phe, His or other L- or D- aromatic amino-acyl residue as such or protected in ester or ether form as above;

such polypeptide being in the above form or modified by isosteric replacement of one or more remaining peptide bonds by reduced, —CH_2—NH—, keto,



hydroxy, —CH(OH)—CH2—, or hydrocarbon, —CH_2—CH_2— isosteric links and further being in free form or in protected form at one or more remaining peptide, carboxyl, amino, hydroxyl or other reactive groups, said process being characterised in that the corresponding individual peptide builder groups are successively reacted singly or as presynth sised units of two or more groups.

2. A process for preparing polypeptide analogues, according to claim 1, of the formula:

X-Y-Pro-Phe-His—A—Val—Z—W (VA)
6 7 8 9 10,11 12 13

where

X, Y, Pro, phe and His are as in claim 1

A is as in claim 1 except that R^1 and R^2, the same or different = ^{t}Bu (isobutyl) or Bzl (benzyl) or other lipophilic or aromatic amino-acid side chain, R^3 = —H; or —SO_2Ph, —$SO_2C_6H_4CH_3$(p), Boc, formyl or other N-protecting group

Z = Tyr, Phe or other L- and D-aromatic amino-acyl residue;

W = —OH as such or in protected ester form as —OR^4 where R^4 = lower alkyl (primarily C_1—C_5 and particularly ^{t}Bu), or Bzl, or other ester forming group; or —NH_2 as such or in protected amide form as —NHR^5 or —$N(R^5)_2$ (R^5 = an N-protecting group e.g. lower akyl as for R^4; $(R^5)_2$ = two such or e.g. cyclo-alkyl, primarily C_3—C_7) or and L- or D- amino-acyl residue e.g. a serine or basic amino-acyl residue as such or in amide form or in protected amide or ester form e.g. containing a group or groups as given for R^4 and R^5 as the case may be; or an amino acid alcohol residue derived therefrom as such or protected in ester or ethere form e.g. containing a group as given for R^4 above or

Z + W = an alcohol derived from Tyr or Phe or other L- or D- aromatic amino acyl residue as such or protected in ester or ether form as above, said process being characterised in that the corresponding individual peptide builder groups are successively reacted singly or as presynthesised units of two or more groups.

3. A process for preparing polypeptide analogues, according to claim 1, of the formula:

$$\underset{6\ 7\ 8\ 9\ 10,11\ 12\ 13}{\text{X-Y-Pro-Phe-His—A—Ile—Z—W}} \qquad \text{(VB)}$$

where

X, y, Pro, Phe and His are as in claim 1

A is as in claim 1 except that R^1 = ^{t}Bu (isobutyl) or Bzl (benzyl) or other lipophilic or aromatic amino-acid side chain, R^2 = ^{i}Pr (isopropyl), and R^3 = H; or —SO_2Ph, —$SO_2C_6H_4CH_3$(p), Boc, formyl or other N-protecting group

Z is as in claim 1

W is as in claim 2 or

Z + W = an alcohol derived from the aromatic residues specified for Z in claim 1, as such or protected in ester or ether form as specified therein, said process being characterised in that the corresponding individual peptide builder groups are successively reacted singly or as presynthesised units of two or more groups.

4. A process for preparing polypeptide analogues according to any one of claims 1 to 3, characterised by isosteric replacement, as set out therein, at one or both of the Pro-Phe or Phe-His links.

5. A process for preparing polypeptide analogues according to any one of claims 1 to 4, characterised by the isosteric replacement at least at the 10,11 position being of the "reduced" kind.

6. A process for preparing polypeptide analogues according to any one of claims 1 to 4, characterised by the isosteric replacement at least at the 10,11 position being of the "hydroxy" kind.

7. A process for preparing the compound:

H-His-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH or

H-Pro-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH or

H-His-Pro-Phe-His-Leu-reduced (SO_2Ph)-Leu-Val-Tyr-OH or

H-DHis-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH or

H-His-Pro-Phe-His-Leu-reduced-Val-Ile-His-OH or

H-DHis-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-OH or

H-DHis-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH or

H-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-OH or

H-His-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH or

H-His-Pro-Phe-His-Leu-reduced-Val-Ile-Tyr-OH or

H-Pro-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-Lys-OH or

H-His-Pro-Phe-His-Leu-reduced-Val-Val-Tyr-OH or

H-His-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH or

H-Pr -Phe-His-L u-hydroxy-Leu-Val-Tyr-OH or

H-DHis-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH or

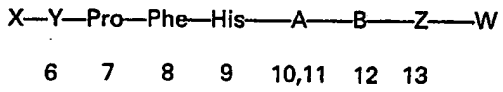
H-His-Pro-Phe-His-Leu-hydroxy-Val-Ile-His-OH or

H-His-Pro-Phe-His-Leu-keto-Val-Ile-His-OH

said process being characterised in that the corresponding individual peptide builder groups are successively reacted singly or as presynthesised units of two or more groups.

Patentansprüche für die Vertragsstaaten: BE CH DE FR GB IT LI LU NL SE

1. Polypeptidanaloges der Formel:



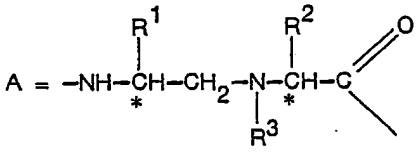
(V)

worin

Pro, Phe und His in substituierter Form vorhanden sein können;

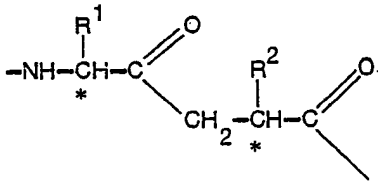
X gleich H ist; oder ein Acyl oder eine andere N-Schutzgruppe, wie Acetyl, Pivaloyl, *t*-Butyloxycarbonyl (Boc), Benzoyl oder Niedrigalkyl (insbesondere C_1—C_5); oder ein L- oder D-Aminoacylrest, welcher selbst ähnlich N-geschützt sein kann, ist.

Y für D- oder L-His oder einen anderen D- oder L- basischen oder aromatischen Aminoacylrest steht, oder abwesend ist;



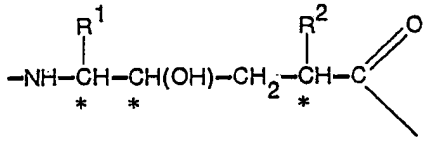
(VI) "reduzierte" Isosterbindung

oder



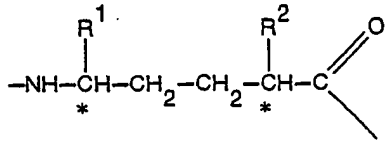
(VII) "Keto-" Isosterbindung

oder



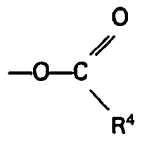
(VIII) "Hydroxy-" Isosterbindung

oder

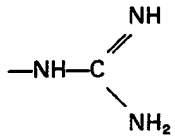


(IX) "Kohlenwasserstoff-" Isosterbindung

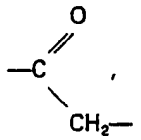
w rin die K nfiguration an asymm trischen Zentren* entwed r R od r S ist, wobei in VIII die Hydroxygrupp als solch od r geschützt in Äther- —OR^4 oder Est r-



Form,
worin R^4 wie unter W unten angegeben ist, anwesend sein kann; und worin
R^1 und R^2 gleich oder verschieden sind und ^{i}Pro (Isopropyl), ^{i}Bu (Isobutyl), Bzl (Benzyl) oder eine andere lipophile oder aromatische Aminosäureseitenkette bedeuten;
R^3 für —H; Niedrigalkyl (C_1—C_5); oder —SO_2Ph, —$SO_2C_6H_4CH_3$(p), Boc, Formyl oder eine andere N-Schutzgruppe steht.
B für D- oder L- Val oder Ile oder einen anderen D- oder L-lipophilen Aminoacylrest steht;
Z für D- oder L- Tyr, Phe, His oder einen anderen L- oder D-aromatischen Aminoacylrest steht; und
W für —OH als solches oder in geschützter Esterform als —OR^4, worin R^4 Niedrigalkyl, in erster Linie C_1—C_5 und insbesondere ^{t}Bu, oder Cycloalkyl, in erster Linie C_3—C_7, oder Bzl, oder eine andere esterbildende Gruppe ist; oder —NH_2 als solches oder in geschützter Amidform als —NHR^5 oder —$N(R^5)_2$ (worin R^5 eine N-Schutzgruppe oder eine andere Substituentengruppe, z.B. Niedrigalkyl, wie für R^4 ist und $(R^5)_2$ = zwei solche, oder z.B. Cycloalkyl, in erster Linie C_3—C_7 darstellt) oder als —NH—$(CH_2)_n$—Q oder —NR^5—$(CH_2)_n$—Q (worin n für 2 bis 6 und Q für NH_2 oder



stehen und worin jeder der an Stickstoff gebundenen Wasserstoffe durch R^5 oder $(R^5)_2$ substituiert sein kann); ein L- oder D- Serin oder Lysin, Arginin oder einen anderen basischen Aminoacylrest als solchen oder in Amidform, substituierter Amidform oder Esterform, z.B. eine Gruppe oder Gruppen enthaltend, wie für R^4 und R^6 oben fallweise angegeben; oder einen davon abgeleiteten Aminoalkoholrest als solchen oder geschützt in Ester- oder Ätherform, z.B. eine Gruppe enthaltend, wie für R^4 oben angegeben; steht oder
Z + W für einen Alkohol, abgeleitet von L- oder D- Tyr, Phe, His oder einem anderen L- oder D-aromatischen Aminoacylrest als solchen oder geschützt in Ester- oder Ätherform wie oben, stehen;
welches Polypeptid in obiger Form oder modifiziert durch isosteren Austausch einer oder mehrerer verbleibender Peptidbindungen durch reduzierte, —CH_2—NH—, Keto-,



Hydroxy-, —CH(OH)—CH_2— oder Kohlenwasserstoff-, —CH_2—CH_2— isostere Bindeglieder vorliegt und weiters in freier Form oder in geschützter Form an einer oder mehreren verbleibenden Peptid-, Carboxyl-, Amino-, Hydroxy- oder anderen reaktiven Gruppen vorliegt.

2. Polypeptidanaloges nach Anspruch 1 mit der Formel

X—Y—Pro—Phe—His——A——Val——Z——W (VA)

6 7 8 9 10,11 12 13

worin
X, Y, Pro, und His wie in Anspruch 1 definiert sind,
A wie in Anspruch 1 definiert ist, ausgenommen, daß R^1 und R^2 gleich oder verschieden sind und ^{i}Bu (Isobutyl) oder Bzl (Benzyl) oder eine andere lipophile oder aromatische Aminosäureseitenkette darstellen,
R^3 für —H oder —SO_2Ph, —$SO_2C_6H_4CH_3$(p), Boc, Formyl oder eine andere N-Schutzgruppe steht;
Z für Tyr, Phe oder einen anderen L- oder D- aromatischen Aminoacylrest steht;
W für —OH als solches oder in geschützter Esterform als —OR^4, worin R^4 Niedrigalkyl (in erster Linie C_1—C_5 und insb sondere ^{t}Bu) od r Bzl, oder eine and re esterbildend Gruppe darstellt; oder für —NH_2 als solches oder in geschützter Amidform als —NHR^5 oder —$N(R^5)_2$ (R^5 ist eine N-Schutzgruppe, z.B. Niedrigalkyl wie für R^4; $(R^5)_2$ b deutet zw i solche oder z.B. Cycloalkyl, in erst r Linie C_3—C_7) oder für einen L- oder D- Aminoacylrest, z.B. einen Serin- oder basischen Aminoacylrest als solchen oder in Amidform oder in geschützter Amidform oder Esterform, z.B. eine Gruppe oder Gruppen enthaltend, wie sie für R^4 und R^5 oben fallweis angegeben sind; oder für einen davon abgeleiteten Aminosäurealkoholrest als

solchen oder geschützt in Ester- oder Ätherform, z.B. eine Gruppe enthaltend, wie für R^4 oben angegeben, steht oder

Z + W einen Alkohol, abgel itet von Tyr oder Phe oder ein n anderen L- oder D- aromatischen Aminoacylrest als solchen oder geschützt in Ester- oder Ätherform wie oben, darstellen.

3. Polypeptidanaloges nach Anspruch 1 mit der Formel

$$X—Y—Pro—Phe—His——A——Ile——Z——W \qquad (VB)$$

$$6 \quad 7 \quad 8 \quad 9 \quad 10,11 \quad 12 \quad 13$$

worin

X, Y, Pro, Phe und His wie in Anspruch 1 definiert sind;

A wie in Anspruch 1 definiert ist, ausgenommen, daß R^1 für ^{t}Bu (Isobutyl) oder Bzl (Benzyl) oder eine andere lipophile oder aromatische Aminosäureseitenkette steht; R^2 für ^{i}Pr (Isopropyl) steht; und R^3 für —H oder $—SO_2Ph$, $—SO_2C_6H_4CH_3(p)$, Boc, Formyl oder eine andere N-Schutzgruppe steht;

Z wie in Anspruch 1 definiert ist;

W wie in Anspruch 2 definiert ist; oder

Z + W für einen Alkohol, abgeleitet von den in Anspruch 1 für Z spezifisch angeführten aromatischen Resten als solchen oder geschützt in Ester- oder Ätherform, wie hierin spezifiziert, stehen.

4. Polypeptidanaloges nach einem der Ansprüche 1 bis 3, modifiziert durch isosteren Austausch, wie hierin dargelegt, an einem oder beiden Pro-Phe oder Phe-His Bindegliedern.

5. Polypeptidanaloges nach einem der Ansprüche 1 bis 4, worin der isostere Austausch an mindestens der Position 10,11 der "reduzierten" Art entspricht.

6. Polypeptidanaloges nach einem der Ansprüche 1 bis 4, worin der isostere Austausch an mindestens der Position 10, 11 der "Hydroxy" Art entspricht.

7. Die Verbindung H-His-Pro-Phe-His-Leu-reduziertes-Leu-Val-Tyr-OH.

8. Die Verbindung H-Pro-Phe-His-Leu-reduziertes-Leu-Val-Tyr-OH.

9. Die Verbindung H-His-Pro-Phe-His-Leu-reduziertes (SO_2Ph)-Leu-Val-Tyr-OH.

10. Die Verbindung H-DHis-Pro-Phe-His-Leu-reduziertes-Leu-Val-Tyr-OH.

11. Die Verbindung H-His-Pro-Phe-His-Leu-reduziertes-Val-Ile-His-OH.

12. Die Verbindung H-DHis-Pro-Phe-His-Phe-reduziertes-Phe-Val-Tyr-OH.

13. Die Verbindung H-DHis-Pro-Phe-His-Leu-reduziertes-Phe-Val-Tyr-OH.

14. Die Verbindung H-His-Pro-Phe-His-Phe-reduziertes-Phe-Val-Tyr-OH.

15. Die Verbindung H-His-Pro-Phe-His-Leu-reduziertes-Phe-Val-Tyr-OH.

16. Die Verbindung H-His-Pro-Phe-His-Leu-reduziertes-Val-Ile-Tyr-OH.

17. Die Verbindung H-Pro-His-Pro-Phe-His-Phe-reduziertes-Phe-Val-Tyr-Lys-OH.

18. Die Verbindung H-His-Pro-Phe-His-Leu-reduziertes-Val-Val-Tyr-OH.

19. Die Verbindung H-His-Pro-Phe-His-Leu-Hydroxy-Leu-Val-Tyr-OH.

20. Die Verbindung H-Pro-Phe-His-Leu-Hydroxy-Leu-Val-Tyr-OH.

21. Die Verbindung H-DHis-Pro-Phe-His-Leu-Hydroxy-Leu-Val-Tyr-OH.

22. Die Verbindung H-His-Pro-Phe-His-Leu-Hydroxy-Val-Ile-His-OH.

23. Die Verbindung H-His-Pro-Phe-His-Leu-Keto-Val-Ile-His-OH.

24. Polypeptidanaloges gemäß jeweils einem der Ansprüche 1 bis 23 zur Verwendung bei einem diagnostischen Test für Hoch-Renin-Zustände, da der Blutdruck am stärksten abfällt, wenn der Reninspiegel hoch ist, oder bei einem chirurgischen Prognosetest für reno-vaskuläre Hypertonie (Stenose der Nierenarterie) durch Verabreichung dieses Polypeptidanalogen, gefolgt von Überwachung des Blutdrucks.

25. Polypeptidanaloges gemäß jeweils einem der Ansprüche 1 bis 23 zur Verwendung in der Lang- und Kurzzeitbehandlung von Herzversagen und allen Hypertoniearten, insbesondere jenen, die in Zusammenhang mit hohen Renin-Serumspiegeln stehen, durch Verabreichung einer reninhemmenden Menge dieses Polypeptidanalogen.

26. Polypeptidanaloges gemäß jeweils einem der Ansprüche 1 bis 23 zur Verwendung als Medikament, dessen tägliche Dosierung 0,001 bis 10 mg/kg Körpergewicht, vorzugsweise 0,01 bis 1,0 mg, dieses Polypeptidanalogen beträgt.

27. Polypeptidanaloges gemäß jeweils einem der Ansprüche 1 bis 23 in Form einer Zusammensetzung mit einem pharmazeutisch akzeptablen Verdünnungsmittel oder Träger.

28. Zusammensetzung nach Anspruch 27 in Einheitsdosierungsform, die die in Anspruch 26 dargelegten Mengen des Analogen oder deren Submultiple enthalten.

Patentansprüche für den Vertragsstaat: AT

1. Verfahren zur Herstellung von Polypeptidanalogen mit der Formel:

$$X—Y—Pro—Phe—His——A——B——Z——W \qquad (V)$$

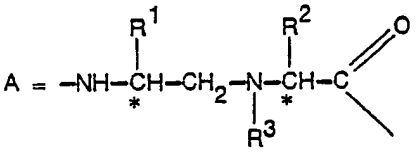
6 7 8 9 10,11 12 13

worin

Pro, Phe und His in substituierter Form vorhanden sein können;

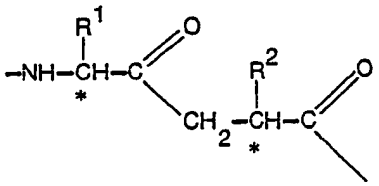
X gleich H ist; oder ein Acyl oder eine andere N-Schutzgruppe, wie Acetyl, Pivaloyl, *t*-Butyloxycarbonyl (Boc), Benzoyl oder Niedrigalkyl (insbesondere C_1—C_5); oder ein L- oder D-Aminoacylrest, welcher selbst ähnlich N-geschützt sein kann, ist.

Y für D- oder L-His oder einen anderen D- oder L- basischen oder aromatischen Aminoacylrest steht, oder abwesend ist;



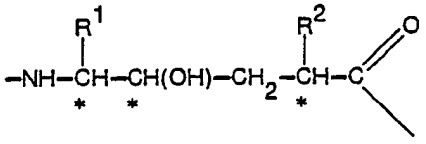
(VI) "reduzierte" Isosterbindung

oder



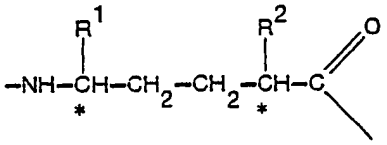
(VII) "Keto-" Isosterbindung

oder



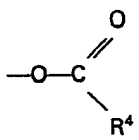
(VIII) "Hydroxy-" Isosterbindung

oder



(IX) "Kohlenwasserstoff-" Isosterbindung

worin die Konfiguration an asymmetrischen Zentren* entweder R oder S ist, wobei in VIII die Hydroxygruppe als solche oder geschützt in Äther- —OR^4 oder Ester-



Form,

worin R^4 wie unter W unten angegeben ist, anwesend sein kann; und worin

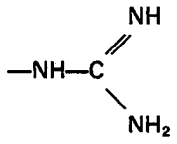
R^1 und R^2 gleich oder verschieden sind und ^{i}Pro (Isopropyl), ^{i}Bu (Isobutyl), Bzl (Benzyl) oder eine andere lipophile oder aromatische Aminosäureseitenkette bedeuten;

R^3 für —H; Niedrigalkyl (C_1—C_5); oder —SO_2Ph, —$SO_2C_6H_4CH_3$(p), Boc, Formyl oder eine andere N-Schutzgruppe steht.

B für D- oder L- Val oder Ile oder einen anderen D- oder L-lipophilen Aminoacylrest steht;

Z für D- oder L- Tyr, Phe, His oder einen anderen L- oder D-aromatischen Aminoacylrest steht; und

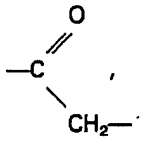
W für —OH als solches oder in geschützter Esterform als —OR^4, worin R^4 Niedrigalkyl, in erster Linie C_1—C_5 und insbesondere ^{t}Bu, der Cycloalkyl, in erster Lini C_3—C_7, oder Bzl, oder eine andere esterbildende Gruppe ist; oder —NH_2 als solches oder in geschützter Amidform als —NHR^5 oder —$N(R^5)_2$ (worin R^5 eine N-Schutzgruppe oder eine andere Substituentengruppe, z.B. Niedrigalkyl, wie für R^4 ist und $(R^5)_2$ = zwei solche, oder z.B. Cycloalkyl, in erster Linie C_3—C_7 darstellt) oder als —NH—$(CH_2)_n$—Q oder —NR^5—$(CH_2)_n$—Q (worin n für 2 bis 6 und Q für NH_2 oder



stehen und worin jeder der an Stickstoff gebundenen Wasserstoffe durch R^5 oder $(R^5)_2$ substituiert sein kann); ein L- oder D- Serin oder Lysin, Arginin oder einen anderen basischen Aminoacylrest als solchen oder in Amidform, substituierter Amidform oder Esterform, z.B. eine Gruppe oder Gruppen enthaltend, wie für R^4 und R^5 oben fallweise angegeben; oder einen davon abgeleiteten Aminoalkoholrest als solchen oder geschützt in Ester- oder Ätherform, z.B. eine Gruppe enthaltend, wie für R^4 oben angegeben; steht oder

Z + W für einen Alkohol, abgeleitet von L- oder D- Tyr, Phe, His oder einem anderen L- oder D-aromatischen Aminoacylrest als solchen oder geschützt in Ester- oder Ätherform wie oben, stehen;

welches Polypeptid in obiger Form oder modifiziert durch isosteren Austausch einer oder mehrerer verbleibender Peptidbindungen durch reduzierte, —CH_2—NH—, Keto-,



Hydroxy-, —CH(OH)—CH_2— oder Kohlenwasserstoff-, —CH_2—CH_2— isostere Bindeglieder vorliegt und weiters in freier Form oder in geschützter Form an einer oder mehreren verbleibenden Peptid-, Carboxyl-, Amino-, Hydroxy- oder anderen reaktiven Gruppen vorliegt, welches Verfahren dadurch gekennzeichnet ist, daß die entsprechenden individuellen Peptidbaugruppen nacheinander einzeln oder als präsynthetisierte Einheiten von zwei oder mehreren Gruppen zur Reaktion gebracht werden.

2. Verfahren zur Herstellung von Polypeptidanalogen nach Anspruch 1 mit der Formel

X—Y—Pro—Phe—His——A——Val——Z——W (VA)

6 7 8 9 10,11 12 13

worin

X, Y, Pro, und His wie in Anspruch 1 definiert sind,

A wie in Anspruch 1 definiert ist, ausgenommen, daß R^1 und R^2 gleich oder verschieden sind und ^{t}Bu (Isobutyl) oder Bzl (Benzyl) oder eine andere lipophile oder aromatische Aminosäureseitenkette darstellen, R^3 für —H oder —SO_2Ph, —$SO_2C_6H_4CH_3$(p), Boc, Formyl oder eine andere N-Schutzgruppe steht;

Z für Tyr, Phe oder einen anderen L- oder D- aromatischen Aminoacylrest steht;

W für —OH als solches oder in geschützter Esterform als —OR^4, worin R^4 Niedrigalkyl (in erster Linie C_1—C_5 und insbesondere ^{t}Bu) oder Bzl, oder eine andere esterbildende Gruppe darstellt; oder für —NH_2 als solches oder in geschützter Amidform als —NHR^5 oder —$N(R^5)_2$ (R^5 ist eine N-Schutzgruppe, z.B. Niedrigalkyl wie für R^4; $(R^5)_2$ bedeutet zwei solche oder z.B. Cycloalkyl, in erster Linie C_3—C_7) oder für einen L- oder D- Aminoacylrest, z.B. einen Serin- oder basischen Aminoacylrest als solchen oder in Amidform oder in geschützter Amidform oder Esterform, z.B. eine Gruppe oder Gruppen enthaltend, wie sie für R^4 und R^5 oben fallweise angegeben sind; oder für einen davon abgeleiteten Aminosäurealkoholrest als solchen oder geschützt in Ester- oder Ätherform, z.B. eine Gruppe enthaltend, wie für R^4 oben angegeben, steht oder

Z + W einen Alkohol, abgeleitet von Tyr der Phe der einen and ren L- oder D- aromatischen Aminoacylrest als solchen oder geschützt in Ester- oder Ätherform wie oben, darstellen; welches Verfahren dadurch gekennzeichnet ist, daß die entsprechenden individuellen Peptidbaugruppen nacheinander einzeln oder als präsynthetisierte Einh iten von zw i der mehrer n Gruppen zur Reaktion g bracht werden.

3. Verfahren zur H rstellung von Polypeptidanalogen nach Anspruch 1 mit d r Formel

X—Y—Pro—Phe—His——A——Ile——Z——W (VB)

6 7 8 9 10,11 12 13

worin

X, Y, Pro, Phe und His wie in Anspruch 1 definiert sind;

A wie in Anspruch 1 definiert ist, ausgenommen, daß R^1 für ^{t}Bu (Isobutyl) oder Bzl (Benzyl) der ein andere lipophile oder aromatische Aminosäureseitenkette steht; R^2 für ^{i}Pr (Isopropyl) steht; und R^3 für —H oder —SO_2Ph, —$SO_2C_6H_4CH_3$(p), Boc, Formyl oder eine andere N-Schutzgruppe steht;

Z wie in Anspruch 1 definiert ist;

W wie in Anspruch 2 definiert ist; oder

Z + W für einen Alkohol, abgeleitet von den in Anspruch 1 für Z spezifisch angeführten aromatischen Resten als solchen oder geschützt in Ester- oder Ätherform, wie hierin spezifiziert, stehen; welches Verfahren dadurch gekennzeichnet ist, daß die entsprechenden individuellen Peptidbaugruppen nacheinander ein zeln oder als präsynthetisierte Einheiten von zwei oder mehreren Gruppen zur Reaktion gebracht werden.

4. Verfahren zur Herstellung von Polypeptidanalogen nach einem der Ansprüche 1 bis 3, gekennzeichnet durch isosteren Austausch, wie hierin dargelegt, an einem oder beiden der Pro-Phe oder Phe-His Bindeglieder.

5. Verfahren zur Herstellung von Polypeptidanalogen nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß der isostere Austausch an mindestens der Position 10,11 der "reduzierten" Art entspricht.

6. Verfahren zur Herstellung von Polypeptidanalogen nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß der isostere Austausch an mindestens der Position 10,11 der "Hydroxy" Art entspricht.

7. Verfahren zur Herstellung der Verbindung H-His-Pro-Phe-His-Leu-reduziertes-Leu-Val-Tyr-OH oder
H-Pro-Phe-His-Leu-reduziertes-Leu-Val-Tyr-OH oder
H-His-Pro-Phe-Leu-reduziertes(SO_2Ph)-Leu-Val-Tyr-OH oder
H-DHis-Pro-Phe-His-Leu-reduziertes-Leu-Val-Tyr-OH oder
H-His-Pro-Phe-His-Leu-reduziertes-Val-Ile-His-OH oder
H-DHis-Pro-Phe-His-Phe-reduziertes-Phe-Val-Tyr-OH oder
H-DHis-Pro-Phe-His-Leu-reduziertes-Phe-Val-Tyr-OH oder
H-His-Pro-Phe-His-Phe-reduziertes-Phe-Val-Tyr-OH oder
H-His-Pro-Phe-His-Leu-reduziertes-Phe-Val-Tyr-OH oder
H-His-Pro-Phe-His-Leu-reduziertes-Val-Ile-Tyr-OH oder
H-Pro-His-Pro-Phe-His-Phe-reduziertes-Phe-Val-Tyr-Lys-OH oder
H-His-Pro-Phe-His-Leu-reduziertes-Val-Val-Tyr-OH oder
H-His-Pro-Phe-His-Leu-Hydroxy-Leu-Val-Tyr-OH oder
H-Pro-Phe-His-Leu-Hydroxy-Leu-Val-Tyr-OH oder
H-DHis-Pro-Phe-His-Leu-Hydroxy-Leu-Val-Tyr-OH oder
H-His-Pro-Phe-His-Leu-Hydroxy-Val-Ile-His-OH oder
H-His-Pro-Phe-His-Leu-Keto-Val-Ile-His-OH,
welches Verfahren dadurch gekennzeichnet ist, daß die entsprechenden individuellen Peptidbaugruppen nacheinander einzeln oder als präsynthetisierte Einheiten von zwei oder mehreren Gruppen zur Reaktion gebracht werden.

Revendications pour les Etats contractants: BE CH DE FR GB IT LI LU NL SE

1. Homologue de polypeptide de formule:

X—Y—Pro—Phe—His——A——B——Z——W (V)

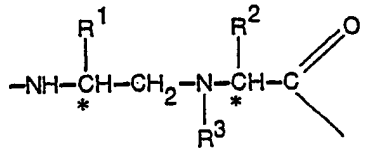
6 7 8 9 10,11 12 13

dans laquelle:

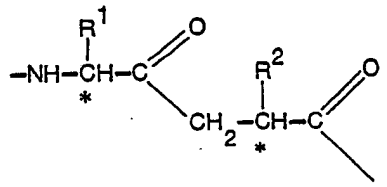
Pro, Phe et His peuvent être sous forme substituée;

X représente H ou un groupe acyle ou autre groupe de protection de l'azote, par exemple un groupe acétyle, pivaloyle, t-butyloxycarbonyle (Boc), benzoyle ou alkyle inférieur (principalement C_1—C_5), ou un reste L- ou D- aminoacyle, qui peut lui-même être protégé de la même façon sur l'azote;

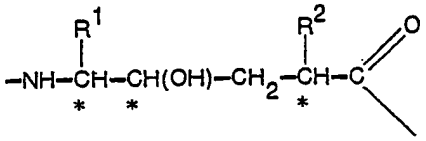
Y représente D- ou L-His ou un autre reste D- ou L-aminoacyle basique ou aromatique, ou est absent;

A représente  (VI) liaison isostère de type "réduit"

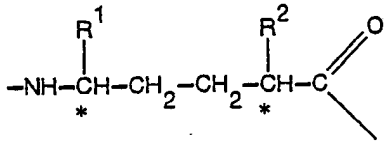
ou

 (VII) liaison isostère de type "cétonique"

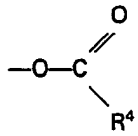
ou

 (VIII) liaison isostère de type "hydroxylé"

ou

 (IX) liaison isostère de type "hydrocarboné"

où la configuration des centres assymétriques est R ou S, où en VIII le groupe hydroxyle peut être présent tel quel ou protégé sous forme d'éther —OR^4 ou d'ester



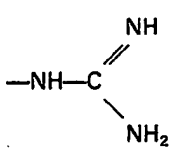
dans lequel R^4 est comme indiqué sous W ci-après, et où R^1 et R^2 identiques ou différents, représentent ^{i}Pro (groupe isopropyle), ^{i}Bu (groupe isobutyle), Bzl (groupe benzyle) ou une autre chaîne latérale amino-acide lipophile ou aromatique,

R^3 représente —H; un groupe alkyle inférieur (en C_1 à C_5); ou —SO_2Ph, —$SO_2C_6H_4CH_3$(p), Boc, un groupe formyle ou un autre groupe de protection de l'azote;

B représente D- ou L- Val ou Ile ou un autre reste D- ou L- amino-acyle lipophile;

Z représente un groupe D- ou L- Tyr, Phe, His ou un autre reste L- ou D- amino-acyle aromatique; et

W représente —OH tel que ou protégé sous forme d'ester —OR^4, où R^4 est un groupe alkyle inférieur, principalement en C_1 à C_5 et en particulier ^{t}Bu, ou un groupe cycloalkyle principalement en C_3—C_7 ou Bzl, ou un autre groupe formant un ester; ou représente —NH_2 tel quel ou en donnant une forme amide protégée représente NHR^5 ou —$N(R^5)_2$ (où R^5 est un groupe de protection de l'azote ou un autre substituant, par exemple un groupe alkyle inférieur comme pour R^4, et $(R^5)_2$ représente deux groupes de ce genre ou, par exemple, un groupe cycloalkyle, principalement en C_3—C_7) ou encore —NH—$(CH_2)_n$—Q ou —NR^5—$(CH_2)_n$—Q (où n est 2 à 6 et Q représente NH_2 ou

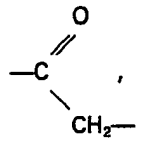


avec n'importe lequel des atomes d'hydrogène fixés sur l'azote pouvant être substitué par R^5 ou $(R^5)_2$); X représente un rest L- ou D-sérin ou lysine, arginine ou un autr reste amino-acyle basique tel quel ou sous forme d'amide, d'amide substitué ou d'ester, contenant par exemple un ou des groupes tels qu'indiqués pour R^4 et R^5 ci-d ssus selon l cas; ou représente un reste amino alcool qui en dérive, tel quel

ou protégé sous form d'ester ou d'éther, contenant par ex mple un groupe tel qu'indiqué pour R^4 ci-dessus, ou

Z + W représentent un alcool dérivé de L- ou D-Tyr, Phe, His ou d'un autre reste L- ou D-aminoacyle aromatique tel quel ou protégé sous forme d'ester ou d'éther comme ci-dessus;

un tel polypeptide étant sous la forme ci-dessus ou étant modifié par remplacement isostérique d'une ou plusieurs des liaisons peptidiques r stantes par les liais ns isostériques qui sont la liaison réduit , $—CH_2—NH—$, cétonique,



hydroxyle, $—CH(OH)—CH_2—$, ou hydrocarbonée, $—CH_2—CH_2—$, et étant de plus sous forme libre ou sous forme protégée sur un ou plusieurs des groupes restants peptides, carboxyles, aminos, hydroxyles ou autres groupes réactifs.

2. Homologue de polypeptide selon la revendication 1, de la formule:

$$X—Y—Pro—Phe—His——A——Val——Z——W \qquad (VA)$$

$$6 \quad 7 \quad 8 \quad 9 \quad 10,11 \quad 12 \quad 13$$

dans laquelle:

X, Y, Pro, Phe et His sont comme à la revendication 1

A est comme à la revendication 1, sauf que:

R^1 et R^2, identiques ou différents, représentent ^{t}Bu (groupe isobutyle) ou Bzl (groupe benzyle) ou un autre chaîne latérale amino-acide lipophile ou aromatique,

R^3 représente —H; ou $—SO_2Ph$, $—SO_2C_6H_4CH_3(p)$, Boc, un groupe formyle ou un autre groupe de protection de l'azote;

Z représente Tyr, Phe, ou un autre reste L- ou D- amino-acyle aromatique;

W représente —OH tel quel ou protégé sous forme d'ester $—OR^4$, où R^4 est un grope alkyle inférieur, (principalement en $C_1—C_5$ et en particulier ^{t}Bu), ou Bzl, ou un autre groupe donnant un ester; ou représente $—NH_2$ tel quel ou en donnant une forme amide protégée représente $—NHR^5$ ou $—N(R^5)_2$ (R^5 représente un groupe de protection de l'azote, par exemple un groupe alkyle inférieur comme pour R^4; $(R^5)_2$ représente deux groupes de ce genre ou, par exemple, un groupe cycloalkyle, principalement en $C_3—C_7$) ou représente un reste L- ou D- amino-acyle, par exemple un reste sérine ou amino-acyle basique tel quel ou sous forme amide ou sous forme amide protégé ou ester, contenant par exemple un ou des groupes tels qu'indiqués pour R^4 et R^5 ci-dessus selon le cas; ou représente un reste alcoolique, dérivé des amino-acides précédents, tel quel ou protégé sous forme d'ester ou d'éther contenant par exemple un groupe tel qu'indiqué pour R^4 ci-dessus, ou bien

Z + W représentent un alcool provenant de Tyr ou Phe ou d'un autre reste L- ou D- amino-acyle aromatique tel quel ou protégé sous forme d'ester ou d'éther comme ci-dessus.

3. Analogue de polypeptide, selon la revendication 1, de formule:

$$X—Y—Pro—Phe—His——A——Ile——Z——W \qquad (VB)$$

$$6 \quad 7 \quad 8 \quad 9 \quad 10,11 \quad 12 \quad 13$$

dans laquelle:

X, Y, Pro, Phe et His sont comme à la revendication 1,

A est comme à la revendication 1, sauf que:

R^1 représente ^{t}Bu (isobutyle) ou Bzl (benzyle) ou une autre chaîne latérale amino-acide lipophile ou aromatique,

R^2 représente ^{i}Pr (isopropyle), et

R^3 représente —H, ou $—SO_2Ph$, $—SO_2C_6H_4CH_3(p)$, Boc un groupe formyle ou un autre groupe de protection de l'azote,

Z est comme à la revendication 1,

W est comme à la revendication 2, ou

Z + W représentent un alcool dérivé des restes aromatiques spécifiés pour Z à la revendication 1, tel quel ou protégé sous forme d'ester ou d'éther, comme spécifié à la revendication 1.

4. Homologue de polypeptide selon l'une quelconque des revendications 1 à 3, modifié par remplacement isostérique, comme indiqué dans celles-ci, pour une des liais ns Pro-Phe u Phe-His ou pour les deux.

5. Homologue de polypeptide s lon l'un quelconque des revendicati ns 1 à 4, dans lequel il y a

remplacement isostérique au moins à la position 10, 11, du type "réduit".

6. Homologue d polypeptide selon l'une quelconqu d s r v ndicati ns 1 à 4, dans lequ l il y a un remplacement isostérique au moins à la position 10, 11 du type "hydroxylé".

7. Le composé: H-His-Pro-Phe-His-Leu-Leu-réduit -Val-Tyr-OH.

8. Le composé: H-Pro-Phe-His-Leu-L u-réduit -Val-Tyr-OH.

9. Le composé: H-His-Pro-Phe-His-Leu- (SO_2Ph)-r´duit -Leu-Val-Tyr-OH.

10. Le composé: H-DHis-Pro-Phe-His-Leu-Leu-réduit -Val-Tyr-OH.

11. Le composé: H-His-Pro-Phe-His-Leu-Val-réduit -Ile-His-OH.

12. Le composé: H-DHis-Pro-Phe-His-Phe-Phe-réduit -Val-Tyr-OH.

13. Le composé: H-DHis-Pro-Phe-His-Leu-Phe-réduit -Val-Tyr-OH.

14. Le composé: H-His-Pro-Phe-His-Phe-Phe-réduit -Val-Tyr-OH.

15. Le composé: H-His-Pro-Phe-His-Leu-Phe-réduit -Val-Tyr-OH.

16. Le composé: H-His-Pro-Phe-His-Leu-Val-réduit -Ile-Tyr-OH.

17. Le composé: H-Pro-His-Pro-Phe-His-Phe-Phe-réduit -Val-Tyr-Lys-OH.

18. Le composé: H-His-Pro-Phe-His-Leu-Val-réduit -Val-Tyr-OH.

19. Le composé: H-His-Pro-Phe-His-Leu-hydroxylè-Leu-Val-Tyr-OH.

20. Le composé: H-Pro-Phe-His-Leu-hydroxylè-Leu-Val-Tyr-OH.

21. Le composé: H-DHis-Pro-Phe-His-Leu-hydroxylè-Leu-Val-Tyr-OH.

22. Le composé: H-His-Pro-Phe-His-Leu-hydroxylè-Val-Ile-His-OH.

23. Le composé: H-His-Pro-Phe-His-Leu-cétonique-Val-Ile-His-OH.

24. Homologue de polypeptide selon l'une quelconque des revendications 1 à 23, pour le diagnostic des taux élevés de rénine, la pression sanguine tombant beaucoup quand le taux de rénine est élevé, ou pour un pronostic chirurgical de l'hypertension réno-vasculaire (sténose de l'artère rénale) par administration dudit homologue de polypeptide suivie d'une surveillance de la pression sanguine.

25. Homologue de polypeptide selon l'une quelconque des revendications 1 à 23, destiné à servir au traitement à long terme et à court terme des défaillances cardiaques et de toutes les formes d'hypertension, en particulier celles associées à des taux élevés de rénine dans le sang, par administration d'une quantité suffisante pour inhiber la rénine, dudit homologue de polypeptide.

26. Homologue de polypeptide selon l'une quelconque des revendications 1 à 23, en tant que medicament à une posologie de 0,001 à 10 mg/kg de poids corporel par jour, de préférence de 0,01 à 1,0 mg.

27. Homologue de polypeptide selon l'une quelconque des revendications 1 à 23, sous forme d'une composition avec un diluant, ou un véhicule pharmaceutiquement acceptable.

28. Composition selon la revendication 27, sous forme de dose unitaire contenant les quantités de l'homologue indiquées à la revendication 26, ou un de leurs sous-multiples.

Revendications pour l'Etat contractant: AT

1. Procédé de préparation d'homologues de polypeptides de formule:

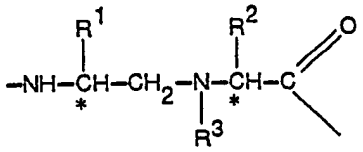
$$\underset{6}{X}—\underset{7}{Y}—\underset{8}{Pro}—\underset{9}{Phe}—\underset{10,11}{His}——A——\underset{12}{B}——\underset{13}{Z}——W \qquad (V)$$

où:

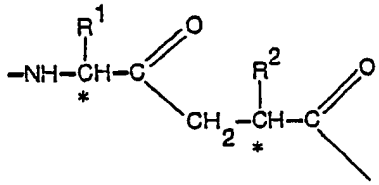
Pro, Phe et His peuvent être sous forme substituée;

X représente H ou un groupe acyle ou un autre groupe de protection de l'azote, par exemple acétyle, pivaloyle, *t*-butyloxycarbonyle (Boc), benzoyle ou alkyle inférieur (principalement C_1—C_5), ou un reste L- ou D- amino acyle, qui peut lui-même être protégé de la même façon sur l'azote;

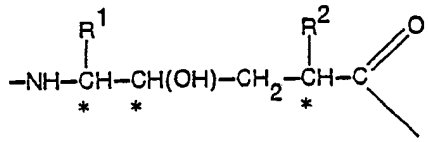
Y représente D- ou L-His ou un autre reste D- ou L-aminoacyle basique ou aromatique, ou est absent;

A représente  (VI) liaison isostère de type "réduit"

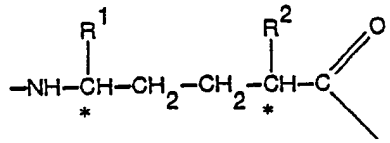
ou

 (VII) liaison isostère de type "cétonique"

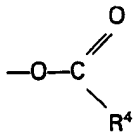
ou

 (VIII) liaison isostère de type "hydroxylé"

ou

 (IX) liaison isostère de type "hydrocarboné"

de telle sorte que la configuration des centres asymétriques soit R ou S, et où dans VIII le groupe hydroxyle peut être présent tel que ou protété sous forme d'éther —OR^4 ou d'ester



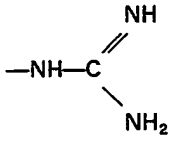
tel que R^4 soit comme indiqué en W ci-dessous, et où R^1 et R^2 identiques ou différents, représentent ^{i}Pro (isopropyle), ^{i}Bu (isobutyle), Bzl (benzyle) ou une autre chaîne latérale aminoacide lipophile ou aromatique,

R^3 représente —H; un groupe alkyle inférieur (C_1—C_5); ou —SO_2Ph, —$SO_2C_6H_4CH_3$(p), Boc, formyle ou un autre groupe de protection de l'azote;

B représente D- ou L- Val ou Ile ou un autre reste D- ou L- aminoacyle lipophile;

Z represénte D- ou L- Tyr, Phe, His ou un autre reste L- ou D- aminoacyle aromatique; et

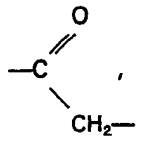
W représente —OH tel que ou protégé sous forme d'ester —OR^4, où R^4 est un groupe alkyle inférieur, principalement en C_1 à C_5 et en particulier ^{t}Bu, ou un groupe cycloalkyle, principalement en C_3 à C_7, ou Bzl, ou un autre groupe formant un ester; ou représente —NH_2 tel que ou en donnant une forme amide protégée représente —NHR^5 ou —$N(R^5)_2$ (où R^5 est un groupe de protection de l'azote ou un autr substituant par exemple un groupe alkyle inférieur comme pour R^4 et $(R^5)_2$ représente deux groupes de ce genre ou, par exemple, un groupe cycloalkyle, principalement en C_3 à C_7) ou encore —NH—$(CH_2)_n$—Q ou —NR^5—$(CH_2)_n$—Q (où n est 2 à 6 et Q représente NH_2 ou



avec n'importe lequel des atomes d'hydr gène fixés sur l'azote pouvant être substitué par R^5 ou $(R^5)_2$); ou représente un reste L- u D- sérine ou lysine, arginine ou un autre reste aminoacyle basique tel quel ou sous forme d'amide, d'amide substitué ou d'ester, contenant par exemple un ou des groupes tel qu'indiqué pour R^4 et R^5 ci-dessus selon le cas; ou représente un reste aminoalcool qui en dérive tel quel ou protégé

sous forme d'ester ou d'éther, contenant par exemple un groupe tel qu'indiqué pour R^4 ci-dessus, ou

Z + W représentent un alcool dérivé de L- ou D-Tyr, Phe, His ou d'un autre reste L- ou D-aminoacyle aromatique tel quel ou protégé sous forme d'ester ou d'éther comme ci-dessus; un tel polypeptide étant sous la forme ci-dessus ou étant modifié par remplacement isostérique d'une ou de plusieurs des liaisons peptidiques restantes par les liaisons isostères qui sont la liaison réduite, $—CH_2—NH—$, c't nique,



hydroxyle, $—CH(OH)—CH_2—$, ou hydrocarbonée, $—CH_2—CH_2—$, et étant de plus sous forme libre ou sous forme protégée sur un ou plusieurs des groupes restants peptide, carboxyle, amino, hydroxyle ou autres groupes réactifs, ledit procédé étant caractérisé en ce que les groupes individuels correspondants constitutifs du peptide sont successivement mis à réagir seuls ou sous forme d'unités pré-obtenues avec deux ou plusieurs groupes.

2. Procédé de préparation d'homologue de polypeptides, selon la revendication 1, de formule:

$$X—Y—Pro—Phe—His——A——Val——Z——W \qquad (VA)$$

$$6 \quad 7 \quad 8 \quad 9 \quad 10,11 \quad 12 \quad 13$$

dans laquelle:

X, Y, Pro, Phe et His sont tels que décrits dans la revendication 1, A est tel qu'à la revendication 1, sauf que:

R^1 et R^2, identiques ou différents, représentent ^{t}Bu (isobutyle) ou Bzl (benzyle) ou une autre chaîne latérale aminoacide lipophile ou aromatique,

R^3 représente —H; ou $—SO_2Ph$, $—SO_2C_6H_4CH_3(p)$, Boc, formyle ou un autre groupe de protection de l'azote;

Z représente Tyr, Phe, ou un autre reste L- ou D- aminoacyle aromatique;

W représente —OH tel quel ou protégé sous forme d'ester $—OR^4$, où R^4 est un groupe alkyle inférieur (principalement en $C_1—C_5$ et en particulier ^{t}Bu), ou Bzl, ou un autre groupe donnant un ester; ou représente $—NH_2$ tel quel ou en donnant une forme amide protégée représente $—NHR^5$ ou $—N(R^5)_2$ (R^5 représente un groupe de protection de l'azote, comme par exemple un alkyle inférieur comme R^4; $(R^5)_2$ représente deux groupes de ce genre ou par exemple un groupe cycloalkyle, principalement en $C_3—C_7$) ou représente un reste L- ou D- aminoacyle, par exemple un reste sérine ou aminoacyle basique tel quel ou sous forme amide ou sous forme amide protégé ou ester, contenant par exemple un groupe ou des groupes tels qu'indiqués pour R^4, R^5 ci-dessus selon le cas; ou représente un reste alcoolique dérivé des aminoacides précédents, tel quel ou protégé sous forme d'ester ou d'éther contenant par exemple un groupe tel qu'indiqué pour R^4 ci-dessus, ou bien

Z + W représentent un alcool provenant de Tyr ou Phe ou d'un autre reste L- ou D- aminoacyle aromatique tel quel ou protégé sous forme d'ester ou d'éther comme ci-dessus, ledit procédé étant caractérisé en ce que les groupes individuels correspondants constitutifs du peptide sont mis successivement à réagir un par un ou sous forme d'unités pré-obtenues de deux ou plusieurs groupes.

3. Procédé de préparation d'homologues de polypeptides selon la revendication 1, de formule:

$$X—Y—Pro—Phe—His——A——Ile——Z——W \qquad (VB)$$

$$6 \quad 7 \quad 8 \quad 9 \quad 10,11 \quad 12 \quad 13$$

où X, Y, Pro, Phe et His sont tels qu'indiqués dans la revendication 1

A est tel qu'indiqué à la revendication 1 sauf que

R^1 représente ^{t}Bu (isobutyle) ou Bzl (benzyle) ou une autre chaîne latérale aminoacide lipophile ou aromatique,

R^2 représente ^{i}Pr (isopropyle), et

R^3 représente —H, ou $—SO_2Ph$, $—SO_2C_6H_4CH_3(p)$, Boc, formyle ou un autre groupe de protection de l'azote

Z est tel qu'indiqué à la revendication 1

W est tel qu'indiqué à la revendication 2 ou

Z + W représentent un alcool dérivé des restes aromatiques spécifiés pour Z à la revendication 1, tel quel ou protégé sous forme d'ester ou d'éther, comme spécifié à la revendication 1, ledit procédé étant caractérisé en ce que les groupes individuels correspondants constitutifs du peptide sont successivement mis à réagir seuls ou sous forme d'unités préobtenues de deux ou plusieurs groupes.

4. Procédé de préparation d'homologues de polypeptides selon l'une quelconque des revendications 1

à 3, caractérisé en ce qu'on effectue un remplacement isostérique comme indiqué dans celles-ci, à l'une des liaisons Pro-Phe ou Phe-His ou aux deux.

5. Procédé de préparation d'homologues de polypeptides selon l'une quelconque des revendications 1 à 4, caractérisé en ce qu'on effectue un remplacement isostérique au moins à la position 10, 11 et de type "réduit".

6. Procédé de préparation d'homologues de polypeptides selon l'une quelconque des revendications 1 à 4, caractérisé en ce qu'on effectue un remplacement isostérique au moins à la position 10, 11 et du type "hydroxylé".

7. Procédé de préparation du composé: H-His-Pro-His-Leu-réduit-Leu-Val-Tyr-OH ou
H-Pro-Phe-His-Leu-réduit-Leu-Val-Tyr-OH ou
H-His-Pro-Phe-His-Leu-réduit (SO_2Ph)-Leu-Val-Tyr-OH ou
H-DHis-Pro-Phe-His-Leu-réduit-Leu-Val-Tyr-OH ou
H-His-Pro-Phe-His-Leu-réduit-Val-Ile-His-OH ou
H-DHis-Pro-Phe-His-Phe-réduite-Phe-Val-Tyr-OH ou
H-DHis-Pro-Phe-His-Leu-réduit-Phe-Val-Tyr-OH ou
H-His-Pro-Phe-His-Phe-réduit-Phe-Val-Tyr-OH ou
H-His-Pro-Phe-His-Leu-réduit-Phe-Val-Tyr-OH ou
H-His-Pro-Phe-His-Leu-réduit-Val-Ile-Tyr-OH ou
H-Pro-His-Pro-Phe-His-Phe-réduit-Phe-Val-Tyr-Lys-OH ou
H-His-Pro-Phe-His-Leu-réduit-Val-Val-Tyr-OH ou
H-His-Pro-Phe-His-Leu-hydroxylé-Leu-Val-Tyr-OH ou
H-Pro-Phe-His-Leu-hydroxylé-Leu-Val-Tyr-OH ou
H-DHis-Pro-Phe-His-Leu-hydroxylé-Leu-Val-Tyr-OH ou
H-His-Pro-Phe-His-Leu-hydroxylé-Val-Ile-His-OH ou
H-His-Pro-Phe-His-Leu-cétonique-Val-Ile-His-OH
ledit procédé étant caractérisé en ce que les groupes individuels correspondants constitutifs du peptid sont mis successivement à réagir seuls ou sous forme d'unités pré-obtenues de deux ou plusieurs groupes.